MONITORING OF FLAVOUR AND FRAGRANCE SUBSTANCES IN SELECTED CONSUMER PRODUCTS AND SUBSTRATES

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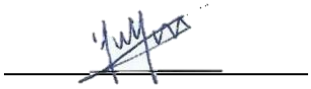
DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety, under the supervision of Assoc. Prof. Liu Shao Quan, in the Food Science and Technology research laboratory (S13-05), Chemistry Department, National University of Singapore, between August 2015 and January 2017.

I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.



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SUMMARY

Flavours and fragrances are an indispensable part of our lives, from the food we eat to the products that we use. Aromas and odours from these products play a large influential role in food flavour perception, consumer choice and quality of life as they help to determine an individual's preferences. Therefore, it is highly rewarding to explore into greater depth of the flavours and fragrances that give rise to the diverse enticing aromas and odours.

Considering the volatile nature of aromas and odours, robust extraction and instrumental methods must be constantly established to achieve reliable analytical results. In the area of volatiles analysis in flavours and fragrances, extraction techniques have gradually shifted from the use of organic solvents to less laborious, solvent-less yet efficient techniques. Indeed, the development of an automated dynamic headspace extraction technique in this study is a representation of such advancements. The use of a sorbent material allows for a large sample amount to be extracted due to its high extraction volume, and analytes of a wide volatility range can be extracted with an array of sorbents currently available. As part of the method development, various extraction parameters were optimised such as the incubation temperature, incubation time, purge gas volume and flow during extraction, as well as the purge gas volume during sample drying. Subsequent sample extractions were performed using the optimised parameters at 30°C for 10.00 min, with a purge gas volume of 600.0 mL at a flow rate of 40.0 mL/min and dried with 840.0 mL of purge gas. These were achieved with subsequent applications to some consumer market products such as shampoo, green tea and milk flavour. This technique demonstrated good calibration linearity with a coefficient of determination of no lesser than 0.9989. Desirable intra-day repeatability was achieved with the highest relative standard deviation of a perfume raw material at 10.4%, and 15.9% for inter-day repeatability. Satisfactory sensitivity was established from a low limit of detection and limit of quantification of 3.6 μg g^{-1} and 11.9 μg g^{-1} respectively.

The technique was further developed into a novel extraction technique for large sample matrices, known as the large volume dynamic headspace

extraction. Considering that there are limited studies on the direct measurement of chemicals interacting and depositing on substrates, this new non-destructive technique provides insights into the feasibility of such analysis. Development of this method included the optimisation of a similar set of extraction parameters, and application to perform a quantitative analysis on the various fragrance volatiles that deposited directly on hair swatches after rinse-off. This method displayed excellent calibration linearity with a coefficient of determination of no lesser than 0.9949 and high sensitivity with the lowest limit of detection and limit of quantification at 0.2 μg g^{-1} and 0.5 μg g^{-1} respectively. Good repeatability was proven by the direct dosing of perfume on the hair swatches instead of after the rinse-off, as sample preparation in terms of washing the hair swatches contributed as a principal source of error for reproducibility. The highest relative standard deviation for samples spiked with 0.004% and 0.008% perfume dosages achieved was 14.8%. The information on the characteristics of fragrance raw materials and their affinities with the substrate acquired could enhance perfume creation knowledge. As a consequence, consumer products with added perfumes possessing longer lasting effects are more likely to be produced, and gain popularity among consumers in future. In short, both the dynamic headspace and large volume dynamic headspace techniques have proven to be efficient and powerful methods for both qualitative and quantitative measurements of volatiles in neat and applied samples respectively. These methods are promising and potentially applicable across various fields other than flavours and fragrances, such as environmental and toxicology.

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LIST OF ABBREVIATIONS

ENLAC	Enzyme-assisted ensiling
LLE	Liquid-liquid extraction
SFE	Supercritical fluid extraction
SD	Steam distillation
SDE	Simultaneous steam distillation-extraction
SAFE	Solvent assisted flavour evaporation
HVT	High vacuum transfer
HS-SPME	Headspace solid phase microextraction
DHS	Dynamic headspace sampling
P&T	Purge and Trap
TD	Thermal Desorption
SBSE	Stir bar sorptive extraction
PDMS	Polydimethylsiloxane
HSSE	Headspace sorptive extraction
DTD	Direct thermal desorption
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
SIM	Selected ion monitoring
GC-O	Gas chromatography-olfactometry
AEDA	Aroma extract dilution analysis
MDGC	Multidimensional GC
DHS-TD-GC-MS	Dynamic headspace-thermal desorption-gas chromatography-mass spectrometry
VOCs	Volatile organic compounds
CIS	Cooled injection system

PRM	Perfume raw material
PTFE	Polytetrafluoroethylene
MSD	Mass selective detector
MPS	Multipurpose sampler
TDU	Thermal desorption unit
PTV	Programmable temperature vaporization
TIC	Total ion chromatogram
RSD	Relative standard deviation
LOD	Limit of detection
LOQ	Limit of quantification
S/N	Signal-to-noise
LSC	Liquid scintillation counting
DART	Direct Analysis in Real Time
XRF	X-ray Fluorescence
LVDHS-TD-GC-MS	Large volume dynamic headspace-thermal desorption-gas chromatography-mass spectrometry

Chapter 1. Introduction and literature review

1.1 Background

Flavours (equivalent to aromas in this context) and fragrances are distinctive odour-active organic chemicals, which play a major role in everyday life through their wide range of applications. Depending on the application as a food flavouring or a perfume, these substances can then be classified as flavours or fragrances respectively.[1] The beginning of the flavour and fragrance industry dates back to the prehistoric period, from developing a liking for a flower scent to enjoying the addition of herbs to food; both of which reflected the better quality of life, despite not being a necessity.[2] Today, the development of new analytical and synthetic chemistry methodologies that allows for the isolation of flavour and fragrance materials in trace amounts[1], along with the growing global economy[2], makes the expansion of this industry even more optimistic than before.

1.2 Flavour and fragrance raw materials

The raw materials used for flavour and fragrance are usually categorised into natural and synthetic. Although the production of synthetic raw materials has expanded with the development of synthetic chemistry techniques, natural raw materials are also used, as synthetic materials cannot reproduce the sensorial features of natural raw materials readily.[3] Also, syntheses of unique flavour and fragrance materials are often not carried out due to economic reasons. Sometimes, the addition of natural substances is mandatory in some flavour productions and in the case of perfumes, there is still a liking for those with natural materials.

Natural ingredients are defined as compounds extracted from natural sources by physical techniques such as distillation and extraction, or by biotechnological processes like enzymatic and microbial reactions.[1, 4] These sources comprise of plants in which all parts could be used, animals, and

microorganisms.[3-4] An example of a plant that produces different raw materials from various plant parts is the bitter orange tree. Its blossoms, leaves and stems, as well as its fruits yield neroli oil, petitgrain oil and bitter orange oil respectively. Linalool, linalyl acetate and limonene can be obtained from these oils.[3] Likewise, the cinnamon tree bark produces cinnamon bark oil, which comprises cinnamaldehyde, whereas the cinnamon leaves provide cinnamon leaf oil that contains eugenol. Animals such as the wild musk deer and civet cat were hunted for their scent glands to garner fragrance materials like muscone and indole, which possess aromatic musk and floral odours.[3-4]

Synthetic flavourants include organic chemicals like alcohols, aldehydes, esters, ketones, lactones that have not been found in nature, and are fit for consumption.[1] Synthetic odorants (known as "nature-identical" previously), on the other hand, include some that had previously been uncovered in natural sources and synthesized after that. Some examples include vanillin, rose oxide and beta-damascone. Interestingly, the starting materials for most synthetic chemicals are hydrocarbon commonly produced from refining petroleum or monoterpenes obtained from turpentine.[4] Synthetics are more frequently applied in fragrances where they are used for their nature-like properties, while natural raw materials are favoured in flavours due to their refined taste and aroma. Many chemical reactions are involved in creating synthetics, such as the catalytic conversions of terpenes into fine chemicals through oxidation, isomerisation, cyclisation and many more. An illustration is the isomerisation of α-pinene to camphene in the production of camphor.[5]

1.2.1 Biosynthesis of plant volatiles

Natural raw flavour and fragrance materials are obtained from their sources through various processes. Before these materials are produced, they often act as secondary metabolites in the form of glycosides within the plant, which can be liberated by enzymatic reactions that cleave the glycosidic bonds.[3] Notably, plant cells, enzymes and microorganisms are important sources to generate flavours biotechnologically as complex flavours can be formed simply from carbohydrates and amino acids. Terpenoids, shikimic acid derivatives, polyketides and alkaloids are some perfume

materials produced through biosynthesis, a metabolic process.[6] Some raw materials such as aromatic methyl ketones are generated from milk fats when milk is converted into cheese in enzymatic and microbial reactions.[3] Some advantages of enzyme biotransformation of plant volatiles include high purity products, excellent selectivity and inexpensive reactants.

Vanillin, which is widely used as a flavour and fragrance ingredient as well as for pharmaceuticals, can now be produced by biotechnology-based approaches. These consist of the bioconversions of ferulic acid, eugenol, metabolism of aromatic amino acids and the application of microorganisms that are genetically engineered, to name a few. Ferulic acid and eugenol undergo conversion by microogranisms such as *Pycnoporus* mutants and *Arthrobacter* to give vanillin. To increase the yield of vanillin produced in the ferulic acid bioconversion process, a two-step process is adopted whereby ferulic acid is first converted to vanillic acid by *Aspergillus niger* before being reduced to vanillin, by *Pycnoporus cinnabarinus*.[7-8]

The use of enzymatic extraction is increasingly popular to obtain flavourants from plants as they showed improved aroma recovery. The action of hydrolysis of enzymes on the plant cell walls produced larger amounts of flavour components, as they were able to pass through the cell walls more easily.[9] Hydrolases remain the most significant enzyme type for flavour production due to their prevalence in the industry without the need for expensive co-factors.[8] Citrozym CEO, containing cellulases, hemicellulases and pectinases, helped to break down pectin, which made the oil-in-water emulsion present in citrus fruits peel less viscous. This increased the recovery of essential oil while reducing the wastewater production, yet not compromising on the quality of the essential oil.[10] In another study, enzyme-assisted ensiling (ENLAC) based on the conversion of water-soluble carbohydrates into organic acids by the action of lactic acid bacteria, was used to improve the extraction efficiency of lemongrass and lemon eucalyptus essential oils. This was also explained by the action of cell-wall hydrolysing enzymes that activated the partial cell wall hydrolysis.[11]

1.2.2 Isolation of natural plant volatiles

The isolation of natural flavour and fragrance ingredients from their sources is performed mainly by mechanical expression, distillation and solvent extraction.[6, 12] The choice of isolation techniques can affect the qualitative, quantitative and sensory characteristics of the final product. Mechanical expression is a straightforward technique that forces the release of volatiles from its natural source using mechanical pressure.[6] Citrus peel oils are examples of essential oils that undergo expression, and they are known as essential oils due to their high content of very volatile terpene hydrocarbons.[12]

Distillation is another technique used to isolate natural volatiles. In the case of essential oils, some volatiles are only produced from their natural sources under distillation at high temperatures as thermolabile substances are converted. Of the various types, steam distillation is commonly used for the extraction of essential oils such as rosemary[13] and coriander[14]. In the recent years, efforts have been made to use greener isolation techniques compared to the conventional steam distillation. Improved microwave steam distillation was designed and successfully applied to the extraction of essential oils from aromatic plant sources like herbs, spices and flowers. It was proven to be more efficient due to shorter distillation times, lower costs and cleaner processes.[15-16]

Simple solvent extraction is the most essential extraction method for volatiles from natural sources up till today. It is used mainly to separate thermally unstable materials, yield non-volatile substances for fixative purposes and low product amounts such as those from blossoms.[12] Different solvents are utilised for different extractions. Ethanol is used to extract ambreine from the sperm whale to yield ambergris, but it is unsuitable for plant sources due to high water content in plants. In the case of essential oils, benzene was conventionally used but due to toxicity concerns, other solvents such as ether, acetone and ethyl acetate are now used instead.[6]

1.3 Odour research

The human sense of smell might not have been regarded with utmost importance[17], but it is certainly one that should be paid more attention to. Psychologically, smells can have an impact on humans directly by affecting moods, arousing memories and experiences, and are important in food indulgence.[18-19] One benefit in understanding the role of an odour plays in food flavour perception, is that it could help in health improvement and prevention of chronic illnesses. This is made possible by the resulting cognition and emotions formed for foods and beverages.[17] Fragrances have also been described to be a primary driver in consumer choices, thereby influencing purchase decisions in consumer products such as beverages, body care, skin care, household cleaners, coffee and more.[20] Therefore, associations of an odour with past experiences can influence one's preference for it heavily, and determine the quality of life indirectly. [19]

In the area of flavours and fragrances, odour research is essential for various purposes. Significantly, odour has the greatest influence on flavour perception among all senses.[21-22] It is thus noteworthy to establish the correlation between an odour and a food product's freshness[21], as flavour is one attribute that determines food quality.[23] Odour analysis can also be vital in food-safety studies when off-flavours associated with undesirable smells occur.[24] For fragrance, much attention has been placed on the perfume performance in consumer products, and one reason for this is to be economical and valuable for every raw material in the perfume in order to achieve consumer satisfaction.[25] Seeking inspiration to create perfumes and reconstituting some natural raw materials have been made possible with volatiles analysis.[26]

1.4 Physical properties of an odour

Odours function in the arousal of human olfactory receptors through its myriad of natural and synthetic compounds.[27] These organic volatile chemicals that form the complex odour are often in varied concentrations.[19] Contrastingly, there is also a simple odour that is formed by only one organic

volatile chemical. The molecules that contribute to the odour usually possess characteristics such as being small, light, polar and hydrophobic.[28]

1.4.1 Odour volatility

In order for an odour to be detected, one of the most important properties is its volatility, which is commonly measured as vapour pressure. The vapour pressure of a chemical is defined as the pressure observed when it is subjected to vacuum at a constant temperature, and is of the same value in air as well as vacuum.[27] Equation (1) defines an odorant concentration in the gaseous phase at 25°C at equilibrium:

$$\left(\frac{P}{760}\right)\left(\frac{273}{298}\right)\left(\frac{M}{22.4}\right) = (P)(M)(5.38 \times 10^{-5}) g/liter \quad (1)$$

where P is the vapour pressure of the odorant, M is the molecular weight of the odorant, assuming that 1 gram of any gas molecule has a volume of 22.4 liters at 0°C and 760 mm Hg atmospheric pressure.[27] When an odorant forms a liquid solution with water or any solvent, its distribution between the liquid and gaseous phases at equilibrium should be considered. In such situations, the air-water partition coefficient, K_{AW}, is given by equation (2):

$$K_{AW} = \left(\frac{P}{S}\right)(M)(5.38 \times 10^{-5}) \quad (2)$$

where S is the solubility of the odorant when the solvent is saturated.[27] When more than one solvent are used to dissolve different odorants in the same solution, the partition coefficients of each component will change the distributions of the components in the air and liquid phases at equilibrium, affecting the odour quality.[27]

Another property that contributes to an odour's volatility is molecular polarity, which refers to the extent an electronic charge is distributed throughout a molecule.[29] Generally, molecules that display similar intermolecular interactions are highly attracted to each other. Some important interactions in odorants include ion-dipole, dipole-dipole, hydrogen bonding and dispersion forces.[29] Certain functional groups in a molecule, such as those with oxygen, can lower its overall volatility due to polarisation with other

similar molecules through their strong affinities.[30] For instance, strong hydrogen bonding can be formed between the carboxylic groups in dimeric carboxylic acid molecules. Volatility is then lowered, as these bonds are not easily broken. Lower volatility results in higher persistence of a perfume.

1.4.2 Odour persistence

The persistence of a perfume can be affected by several factors in addition to its volatility. In fact, the perfume dosage applied, the interactions between a perfume and the base in which it is added into, as well as the interactions between the perfume and the substrate that it has been applied on, each contributes to the long-lastingness of a perfume.[20] Particularly, the attractive and repulsive forces between a perfume and its substrate, are worth noting to gain a deeper understanding of the perfume behaviour.[17] These forces are also known as forces of adhesion, while the degree of adhesion is termed as substantivity.[20] Some surfaces have higher adsorbing capacity, as compared to others, like activated coconut charcoal, to neutral odorants and metallic surfaces to sulfurous and nitrogenous odourants.[17] Equation (3) governs the relationship between quantity x of an odorant adsorbed by adsorption sites and its concentration C in either a solution or the vapour phase:

$$x = mkC^b \tag{3}$$

m defines the mass of the adsorbing material, k represents a constant and b is always less than unity.[17]

For the purpose of creating a long-lasting perfume, it is then of no surprise that fixation is generally practised. Fixatives are low-volatility, high-boiling materials that reduces the evaporation of more volatile chemicals in the same perfume.[17,20] Due to their low air-oil partition coefficients, these oily or waxy perfume ingredients are able to hold large amounts of fragrance volatiles before liberating them slowly over a period of time.[17] Both natural and synthetic compounds can act as fixatives, with the former possessing superior fixative power, especially musk, civet, castoreum and ambergris.[17]

Interestingly, perfume fixatives have also been reported to increase the protection time of mosquito repellents.[31]

1.5 Flavour and fragrance measurement

Flavour and fragrance analyses are usually made up of sensory and instrumental analysis.[32] Sensory analysis is a multivariate “analytical” (or evaluation) technique whereby panelists rate a flavour as an overall perception of all individual flavours present in the food sample. In contrast, instrumental analysis is a univariate analytical technique since each component is interpreted on its own instead of a combined flavour as a whole.[33]

In sensory analysis, olfactive descriptors are collected/selected to ascertain human perception towards a flavour. It should be noted that different chemical structures or compound mixtures could trigger similar odours, because the same sensory impressions are created by our brain receptors. In contrast, slight molecular structure changes could also cause a large difference in an odour characteristic or intensity.[32] Normally, a professional/trained sensory panel conducts the sensory tests. However, sensory tests can sometimes be costly, time consuming, difficult in gathering a significant sample population and are unable to generate real-time feedback.[32-33]

Instrumental analysis acts as a complementary technique in addition to sensory analysis. The methods utilised in instrumental analysis provide several advantages. Firstly, the identification of materials analytically gives an idea of compounds giving rise to flavour changes, off-flavours and malodours.[32] Such identifications and purity checks determine the satisfactoriness of flavour and fragrance ingredients.[34]

In this research, emphasis will be placed on the instrumental aspect of flavour and fragrance measurements instead of sensory analysis.

1.6 Extraction of flavour and fragrance volatiles

Several common extraction techniques such as solvent extraction, distillation, headspace sampling and sorptive extraction have been adopted to pre-concentrate food aroma and fragrance volatiles before instrumental analysis. A few of these are selected for discussion in this section.

1.6.1 Solvent extraction techniques

1.6.1.1 Liquid-liquid extraction (LLE)

LLE is a simple extraction technique that refers to the extraction of a liquid sample by an immiscible solvent. This is governed by the partition constant of the target analyte between the 2 immiscible solvents.[35] The extraction takes place from the aqueous liquid food phase into the organic phase, followed by the evaporation of the solvent to recover flavour components.[21] It is employed frequently in wine aroma analysis and remains as the reference technique in wine analysis because of its high repeatability, ability to extract a diverse range of volatiles that have high partition coefficients to common organic solvents and to perform simultaneous extractions.[36-38] Some other applications of LLE in food analysis also include the extraction of contaminants in bovine milk, carbohydrates from milk and patulin in apple juice.[35]

1.6.1.2 Supercritical fluid extraction (SFE)

SFE makes use of solvents with a combination of gas and liquid properties. The most commonly used solvent in flavour and fragrance analysis for SFE is carbon dioxide, CO_2, in its supercritical phase. It presents several advantages; it is non-toxic, non-flammable, non-corrosive, chemically stable, low cost and it returns to the vapour phase after extraction such that the target analytes can be isolated easily.[35, 39] Also, it is ideal for selectively dissolving analytes by modifying the temperature and pressure conditions, and by adding organic modifiers.[21] Since its critical point is rather low at 31.1°C, extractions can be conducted under less harsh conditions.[24] Pertaining to fragrance volatiles isolation from flowers, SFE is more useful than distillation techniques that fail to reproduce the odours from the natural

source.[39] Examples of SFE applications include volatiles extraction from spices[40] and roasted peanuts[41].

1.6.2 Distillation techniques

1.6.2.1 Steam distillation (SD)

SD is a conventional way of isolating flavour and fragrance volatiles from food samples and plant parts.[24] Volatiles are released from the sample and collected by the steam that flows through the sample matrix, and subsequently condensed back into the boiler. [24, 42] Since SD uses water as an extracting medium, there is no concern with regards to the usage of organic solvents. Also, it is a low cost and easy technique, and it can be applied to a wide variety of chemicals with different physical properties.[43] However, SD can cause thermal degradation of products, which is unfavourable as the product quality in terms of shelf life and organoleptic properties declines.[21, 43] Furthermore, SD consumes a large amount of energy with the heating of equipments.[21, 43] Lastly, the volatiles collected can be diluted by water when they are accumulated in cold traps.[21] Other than the usual isolation of volatiles from essential oils as previously mentioned, SD has also been applied in food samples such as green tea.[44]

1.6.2.2 Simultaneous steam distillation-extraction (SDE)

Volatiles are extracted from the sample in SDE, also known as the Likens-Nickerson SDE, similarly to SD. Water vapour, the volatiles and an immiscible organic solvent are co-condensed on a cold tube, with each returning to the individual boiling flask where reflux continues.[24, 42] Sample volatiles and the solvent are condensed simultaneously and recycled, while extraction takes place, hence the name SDE.[45] Some advantages of SDE are high recoveries of analytes; single step extraction; small amount of solvent needed; reduced pressure setting that can minimise thermal decomposition and artefacts formation.[21, 45] However, the low pressure might cause difficulty in maintaining the solvent level in the setup.[21] SDE has been extensively used in the extraction of volatiles from essential oils, as well as food and beverages such as prawns and lobsters[46], tea[47], beer[48] and flavouring from rice husk[49].

1.6.2.3 Solvent assisted flavour evaporation (SAFE)

SAFE was developed as an improvement to a previous extraction method based on the high vacuum transfer (HVT) technique.[50] The aim was to achieve a rapid isolation of food volatiles from various food matrices, including both solid and aqueous food. SAFE was proved to be more superior to HVT in terms of volatiles recovery such as the more polar odorants. In fact, it can distill aqueous food directly to produce distillates with volatiles, separating them from the non-volatiles. It is also capable of extracting flavour volatiles from foods with high fat content. Some other advantages are its stable and safe apparatus setup, lower cost, fast extraction time.[50] SAFE is a popular extraction technique used for characterisation and quantification of aroma compounds in food systems such as apple cider[51], baked matrices[52], cooked brown rice[53] and pork broth[54].

1.6.3 Headspace sampling techniques

1.6.3.1 Headspace solid phase microextraction (HS-SPME)

SPME was first developed in 1990, as a rapid, solvent-less technique that utilises a small sorbent volume for sample extraction. This sorbent material can be made of a porous solid sorbent for larger surface area during adsorption, or a heavy polymeric liquid.[55] The working principle of HS-SPME is by the analyte partitioning between the sample headspace and the fibre coating, followed by desorption into an analytical instrument.[56] The extraction efficiency in HS-SPME can be affected by a few factors such as agitation and extraction temperature. As temperature increases, a larger amount of analytes fills the headspace, and the extraction process would be favoured. However, with the increased temperature, the partition coefficient to the fibre coating decreases, which could lead to a reduced amount of analyte extracted at equilibrium. Therefore, the optimisation of agitation, extraction temperature, sample volume, time is vital in HS-SPME.[55] Today, the use of HS-SPME is widely accepted in aroma and fragrance analysis. This could be explained by the numerous advantages that it offers; quick, simple, convenient, no interference by sample matrix.[21, 24] Applications of HS-SPME include extraction of volatiles in leaf samples[57], tobacco products[58], single rice kernels[59] and shampoos[60].

1.6.3.2 Dynamic headspace sampling (DHS)

In DHS, commonly known as purge and trap (P&T), a purified gas flow is continuously purged through the sample matrix to increase extraction of the aroma volatiles. The extracted volatiles are then retained on an adsorbent or a cooled trap. When the extraction process is completed, the trapped volatiles are thermally desorbed by heating the trap for chromatographic analysis.[24, 61-62] Different variations of DHS exist in the trap types and their trapping method, such as on-column vapour traps, cryogenic trapping and trapping on a sorbent material.[21] Among the wide choices of sorbent materials available, Tenax® is used most commonly due to its ability in trapping volatiles of a wide volatility range, stability at high temperatures and low affinity towards water.[62]

The combination of DHS with thermal desorption (TD) provides many advantages over solvent desorption. It is simple, has lower limits of detection, and is not affected by solvent peaks in analysis.[24] Solvent desorption, contrastingly, may cause the loss of very volatile components.[21] Examples of P&T applications include volatile analysis of extra virgin olive oil[63], sweet cream butter[64] and pomegranate juice[65].

1.6.4 Sorptive extraction techniques

1.6.4.1 Stir bar sorptive extraction (SBSE)

The development of SBSE by Baltussen et al.[66] in 1999 was presented as an alternative to SPME. A magnetic stir bar coated with a layer of polydimethylsiloxane (PDMS) is placed in liquid samples and extracts the analytes while it stirs. Following that, it is removed, placed in a glass liner and undergoes TD of the analytes in the analytical instrument. Like SPME, the working principle of SBSE is based on the partitioning between the PDMS coating and the analyte concentration in the aqueous sample phase. This is governed by the partition coefficient and the phase ratio between the 2 phases. The advantages of SBSE over SPME are the increase in sensitivity due to the increase in extraction capacity, and no degradation of the PDMS coating observed after 100 extractions.[66] Other advantages are its convenience and rapidness. SBSE has been applied in the analysis of volatiles in Chinese liquors[67], orange juice[68], laundry malodour[69] and recently,

a new multi-SBSE method in the extraction of roasted green tea[70]. SBSE can also be applied in headspace sampling, by a technique known as the headspace sorptive extraction (HSSE).

1.6.4.2 Direct thermal desorption (DTD)

The attractiveness of DTD lies in its simplicity and rapidness. Minimal sample preparation steps are required as samples are placed in a TD liner in between glass wool plugs without any solvent needed. The sample is then heated to release the volatile components and desorbed directly for chromatographic analysis.[71] Sometimes, a cryogenic trap is also used to focus analytes before entering the analytical instrument for better peak shape. However, problems can arise in quantitative analysis such as including an internal standard and loss of purged volatiles if a split/splitless injector was used. DTD is suitable for thermally stable and low moisture content samples in powdered form to ensure uniformity and reproducibility in the results obtained.[72] DTD has been applied to extract volatiles from plants[73], olive oil[74] and cheddar cheese[75].

1.7 Instrumental methods

Due to the complex nature of flavours and fragrances, which consist of numerous chemical components of various volatilities, polarities, molecular weights, chemical structures, highly efficient instruments for separation such as the gas chromatography (GC) are often utilised.[76] Moreover, in view of the constant search for new flavours and fragrances molecules today, a continuous effort to develop efficient separation and identification techniques is indispensable.

1.7.1 Gas chromatography-mass spectrometry (GC-MS)

Capillary GC is preferred in volatiles analysis as it can be performed in short analysis time achieving high sensitivity and good separation. The selection of GC columns is based on the polarity of their stationary phase,

mainly non-polar columns based on methyl polysiloxanes and methyl-phenyl-polysiloxanes or polar columns based on polyethyleneglycol.[76] However, the over-reliance on the GC retention time should be avoided since there could be compounds with the same retention time under the same conditions, especially in a low-resolution GC system.[77]

The hyphenated technique GC-MS offers a solution to this. MS is known to be a robust identification technique for chemical structural elucidation as volatiles exhibit characteristic mass spectral fragmentation patterns. The reliability of MS is further proven by its high accuracy in chemical identification for pure compounds.[77] Furthermore, the option of MS detection with selected ion monitoring (SIM) enhances it further as a confirmatory technique, which explains why GC-MS is highly preferred in environmental, toxicological and food analysis.[78]

Occasionally in headspace sampling, the analyte concentration can be very low. Attempts to increase the sample volume or a larger diameter with thicker stationary phase coating GC column may be used to allow more samples to enter the GC column. Unfortunately, this would cause band broadening in the chromatographic peaks, sacrificing the peak resolution. Hence, cryo-trapping of the analytes before they enter the GC column is practised by condensing the volatiles on a cold trap during the transfer of the sample. Subsequently, the trap is rapidly heated to release all analytes into the GC at the same time so that a sharp, resolved peak shape could be obtained. However, care must be taken to avoid ice formation that can block the GC column by removing water present in the sample.[61]

1.7.2 Gas chromatography-olfactometry (GC-O)

GC-O is an aroma evaluation instrumental technique performed in flavour and fragrance analysis. This is done through the combination of olfactometry with GC separation. It relates an aromatic molecule's odour activity in air, while disregarding odourless components, to the eluted chromatographic peak. GC-O is essential for odour research, because it possess the selectivity and sensitivity like the human nose, in addition to the analytical information it provides.[79] Particularly in flavour analysis, it is often

the first step practised.[80] In fragrance analysis, odorants or fragrance molecules in consumer products may be in too low concentrations to be detected by routine GC-MS analysis, which makes GC-O analysis necessary.[81]

The techniques for GC-O can be broadly classified into dilution and time-intensity techniques. Dilution techniques, built on panel assessment of serial diluted aroma extracts until odour is no longer perceived, are more frequently applied.[79] Based on the dilution technique, CharmAnalysis was developed by Acree et al.[82] in 1984. It involved the sensory detection of randomly diluted samples by an assessor from the beginning till the end when the odour was detected. This time frame was recorded, and a graph of the duration against the dilution value was plotted, which is known as the aromagram.[79] A set of standards were then analysed by GC, and their retention times were related to retention indices in the time frame used by the assessor.[82] The chromatographic peak areas reflected the odorant amounts present.[83] A limitation of CharmAnalysis is the requirement of three different trained professionals for performing replicates in quantitative experiments.[79]

Aroma extract dilution analysis (AEDA) developed by Grosch, on the other hand, is a quantitative GC-O technique where assessors are presented with samples of ascending dilution order.[79-80] Odorants with the highest dilution factors were quantified for their concentration and odour activity values. The results were also presented in an aromagram, in the form of dilution factors or the logarithm of dilution factors against their retention indices.[79] AEDA is not able to account for odorants loss during analytes isolation, and is subjected to the ability of each assessor's perception towards the aroma.[80] In contrast, time-intensity measurement is represented by Osme, where an assessor gives a rating of the odour intensity, using a time-intensity instrument, which represents the aroma qualities.[83]

A main strength of GC-O is the detection of unknown aroma active compounds present in low concentrations. However, it also has its drawbacks. GC-O is unable to identify synergistic and antagonistic effects from interactions of other aroma impactful compounds in the same sample.[84] Also, it is time intensive, and requires sniffers to be screened for sensitivity.[85]

1.7.3 Other GC techniques

In the flavour and fragrance field, advancements of GC are desired. Besides GC-MS and GC-O, there are other GC techniques that are applied for efficient analysis. One of them is the fast-GC, which targets to shorten analysis time without compromising on accurate qualitative and quantitative data.[76] An approach developed by Bicchi et al.[86] through the use of shorter GC columns with conventional inner diameters had proven successfully in its fast analysis of rosemary and chamomile essential oils. The other approach by Mondello et al.[87] was also shown to be effective in its fast analysis of citrus essential oils by using short GC columns with narrower inner diameters, under harsher conditions such as increased pressure of the GC inlet, split ratios, and higher temperature ramp rates.

Another popular technique is the multidimensional GC (MDGC) through heart-cutting, where analytes that are not separated satisfactorily on the first GC column are transferred to a second GC column coated with a stationary phase of a different characteristic.[88] Examples of MDGC applications include the identification of volatiles in malt whisky[89], Japanese green tea[90] and essential oils[91].

1.8 Thesis outline

The objectives of this research work revolved around new methods development for the identification and quantification of flavour and fragrance volatiles. This thesis begins with a general introduction of flavour and fragrance materials, odour research and its physical properties, as well as a literature review on common extraction and instrumental methods used in flavour and fragrance analysis.

Chapters 2 and 3 denote/present the detailed methodologies developed and the results obtained. Discussions on the analysis results achieved are also shown. Chapter 2 focuses on volatiles analysis in consumer products while Chapter 3 offers an insight into volatiles deposition on substrates.

Last but not least, this thesis ends with a concluding chapter summarising all the research that has been conducted, some recommendations and future work that can be explored.

Chapter 2. Monitoring of flavour and fragrance volatiles in selected consumer products via automated dynamic headspace-thermal desorption-gas chromatography-mass spectrometry (DHS-TD-GC-MS)

2.1 Introduction

Flavours and fragrances are common additives in consumer products today. They are complicated mixtures made up of many different volatile chemicals in minute amounts, ranging from parts per million to parts per trillion.[39] The power of these additives must not be underestimated as they strongly influence consumers' preference for a product. In fact, a fragrance can be used to differentiate a product from another, by assigning it with distinct characteristics, which are representative of the product performance.[92] Likewise, a flavour, by contributing as the main sensorial feature of a food product, dictates its uniqueness and acceptability.[72, 93]

In order for a product to be outstanding, flavour and fragrance analyses are necessary in addition to consumer evaluation.[92] Any malodour would probably result in a negative impression of the product. Particularly in modern flavour analysis, the interpretation of a complete flavour profile helps to identify attractive flavour compounds that are stronger organoleptically as compared to others.[39] Both qualitative and quantitative information can also improve the management of raw materials' quality through selection of target compounds.[93]

2.1.1 Challenges in flavour and fragrance volatiles analysis

Sometimes, difficulties arise in volatiles analysis due to the sample nature. For instance, the complex matrix of shampoo causes challenges in the quantification of perfume materials in shampoos, which is due to strong interactions between the analytes and shampoo base.[60] Furthermore, since shampoos are highly viscous, mass transfer takes a longer time, leading to longer extraction timings.[60] This spurred the work of Debonneville and

Chaintreau[94] in an online clean-up of volatile compounds in such complex matrices, to obtain a clean separation of volatiles from non-volatiles before instrumental analysis. Fragrance analytes were first deposited on a PDMS foam before being transferred to a secondary trapping material, and injecting into the GC column later on.

In food samples, detection can be a problem when aroma analytes are present in very low concentrations. This is not uncommon, since some odorant concentrations can even be present at lesser than 1 ng L^{-1} of the substance.[24] In other examples, volatiles in wines[95], honey[96] and corn tortilla chips[97] were present in µg L^{-1}, ng g^{-1} and µg kg^{-1} respectively. Furthermore, much effort is needed in odour identification and quantification since food aromas are formed through complex combinations of ultra-trace odorants from diverse classes.[21, 24] As an illustration, 100 volatile compounds were characterised in 22 commercial honey samples from various sources, with 18 of them being identified for the first time.[96] In Bordeaux Cabernet Sauvignon and Merlot wine extracts, 48 volatiles were identified using the AEDA method.[98] For the above-mentioned reasons, highly sensitive analytical procedures ought to be developed for the detection and quantification of such volatiles.

2.1.2 Comparison of DHS against other extraction techniques in flavour and fragrance volatiles analysis

A crucial aspect in volatiles analysis such as that in food aroma analysis is to extract and isolate target compounds quantitatively yet restrict the amount of artefacts produced.[21] Therefore, not all extraction methods are suitable to be utilised despite the wide range of extraction techniques available.

Although LLE is a simple technique traditionally used in volatiles extraction, the use of organic solvents and their evaporation leads to product degradation or even loss[37], which is especially undesirable in trace analysis. Other than the loss of analytes, purification is also needed, as there is poor selectivity of volatiles from other components in the sample.[60] Besides, LLE is also limited by low concentrations of analytes in foods.[21] The move away from

LLE in the recent years can be explained by the environmental and health concerns when organic solvents are involved.[21, 24]

Moving towards solvent-less methods, SPME is one simple and rapid extraction technique frequently used in flavour and fragrance volatiles analysis over the past two decades.[24] Perfume compounds in shampoos have been extracted using SPME. To increase the amount of analytes extracted, shampoos have been diluted with water, which reduced shampoo viscosity. As for small sample amounts, exhaustive SPME extraction was used.[60] Moving away from the traditional SPME, a miniaturised and automated internally cooled SPME fibre device was invented to increase extraction efficiency for the quantification of fragrance analytes.[99] The working principle of this device was based on the concurrent sample heating and fibre coating cooling.[36] However, SPME has its deficiencies. Due to the thin polymeric fibre coating, it is unable to adsorb large quantities of volatile organic compounds (VOCs) since it has a limited fibre volume.[100] The fibre degrades with time and it can usually be used for only about 50 sampling/reconditioning cycles.[100] Furthermore, there is no protection of the polymer coating and the fused-silica used for the fibre is fragile.[100] The distribution coefficient and amount of analytes adsorbed on the fibre also vary with any modification in experimental conditions.[101] With such sensitivity, the technique's reproducibility would be adversely affected.

Another popular technique employed in the extraction of flavour and fragrance volatiles is SBSE. Despite its many advantages such as being a solvent-less miniaturised technique, there are also factors affecting the performance of SBSE. During extraction, an equilibrium can be achieved faster with increased temperature but efficiency decreases as the distribution coefficient of the analytes drops.[102] At temperatures higher than 40°C, the lifetime of the PDMS extraction phase on the stir bar could be reduced.[102] Due to mechanical stirring as the stir bar comes into contact with the sample in a sample vial, deterioration in the physical condition of the PDMS coating could arise with faster stirring speed.[102] Comparing against headspace techniques, SBSE is more invasive as it comes into direct contact with the sample itself.[95] Lastly, SBSE cannot be fully automated as the removal, rinsing and drying of stir bars are usually performed manually and could also be possible error sources.[103]

On the other hand, the advanced automated DHS solvent-less extraction technique has been increasingly applied in volatile analysis in environmental, food and fragrance samples due to its low detection limits, high sensitivity and easy sample preparation steps.[104] TD tubes are packed with a suitable sorbent material, which are used to capture and concentrate VOCs purged from the sample headspace using nitrogen gas. After the trapping stage, the loaded TD tube can be dried under a flow of gas to remove any moisture trapped from liquid samples (**Figure 2.1**). The analytes are then thermally desorbed and cryo-focused in a cooled injection system (CIS) before entering the GC. An edge over SPME, DHS allows for larger amounts of analytes to be extracted due to its greater capacity of the sorbent material.[104] Moreover, a broader range of analytes with different chemical properties can now be detected given the availability of single and multiple sorbent materials.[104-105] DHS has also been reported to show good precision and repeatability, especially due to the ability for automation.[95]

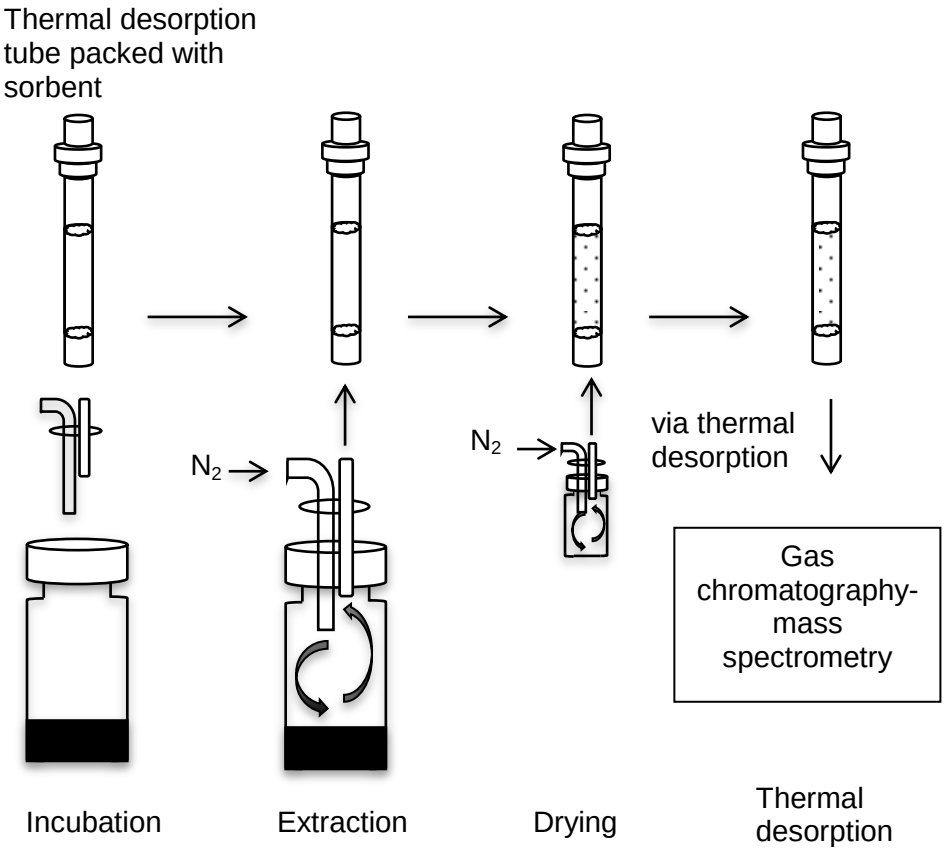

Figure 2.1 Schematic diagram of dynamic headspace (DHS) sampling.

2.1.3 Objective

In this study, a method based on the automated DHS-TD-GC-MS was developed for the quantification of perfume VOCs. As the extraction yield could be affected by the DHS incubation temperature, incubation time, extraction volume, extraction flow, sorbent material and drying volume, these parameters ought to be optimised to ensure accuracy in results.[105-106] Adopting the optimised parameters, the method was applied in the quantitative analysis of neat shampoo samples spiked with perfume at different dosages. The developed method was also used to quantify flavour volatiles in different food samples.

2.2 Experimental materials and methods

2.2.1 Chemicals and reagents

Perfume A is an in-house perfume that contains the following perfume raw materials (PRMs) : pipol acetate (0.3% w/w), hexyl acetate (0.7% w/w), limonene (3.5% w/w), melonal (0.4% w/w), dihydromyrcenol (2.1% w/w), zestover (1.2% w/w), linalool (8.5% w/w), benzyl acetate (0.5% w/w), styrallyl acetate (0.4% w/w), undecavertol (1.6% w/w), verdox (4.2% w/w), benzyldimethylcarbinol acetate (1.1% w/w), allyl cyclohexylpropionate (1.6% w/w), benzyldimethylcarbinol butyrate (2.3% w/w), lilial (9.2% w/w), iso e super (6.7% w/w) and hexyl salicylate (12.0% w/w). Yara yara and ethyl salicylate are in-house PRMs used as internal standards in parameter optimisation and method validation respectively. The chemical names, chemical formulae, molecular weights, vapour pressures and selected ions for quantification are shown in **Table 2.1**. The unperfumed pearly shampoo base (Base no.: EDL-13-001) is an in-house base. Reagent alcohol and methanol were purchased from Tritech Scientific Pte. Ltd.

The food samples used for flavour volatiles analysis in the later part of method application are Pokka green tea (jasmine flavour) manufactured by Pokka Corporation (S) Ltd and purchased from a local supermarket as well as a milk flavour from Shanghai Xumei Food Tech Co. Ltd.

Table 2.1 Chemical names, chemical formulae, molecular weights, vapour pressures and selected quantifying ions for all perfume raw materials (PRMs). The target ions have been marked in bold.

PRM	Chemical name[a]	Chemical formula[a]	Molecular weight (g mol^{-1})[a]	Vapour pressure (hPa)[a]	Quantifying ions (m/z)
Pipol acetate	3-hexenyl acetate	$C_8H_{14}O_2$	142.20	1.51962	**67**/82
Hexyl acetate	Hexyl acetate	$C_8H_{16}O_2$	144.21	1.93285	**56**/84
Limonene	1,8-p-menthadiene	$C_{10}H_{16}$	136.23	1.93285	**93**/136
Melonal	2,6-dimethyl-5-heptenal	$C_9H_{16}O$	140.22	1.01175	**82**/140
Dihydromyrcenol	2,6-dimethyl-7-octen-2-ol	$C_{10}H_{20}O$	156.27	0.16529	**59**/123
Zestover	2,4-dimethyl-3-cyclohexene-1-carbaldehyde	$C_9H_{14}O$	138.21	0.46922	**107**/123
Linalool	3,7-dimethyl-1,6-octadien-3-ol	$C_{10}H_{18}O$	154.25	0.11091	**71**/93
Benzyl acetate	Benzyl acetate	$C_9H_{10}O_2$	150.17	0.24927	91/**108**
Styrallyl acetate	1-phenylethyl acetate	$C_{10}H_{12}O_2$	164.20	0.14930	**104**/122
Undecavertol	4-methyl-3-decen-5-ol	$C_{11}H_{22}O$	170.29	0.00793	**99**/141

Table 2.1 (Cont'd)

Verdox	2-tert-butylcyclohexyl acetate	$C_{12}H_{22}O_2$	198.30	0.07105	**82**/123
Benzyldimethylcarbinol acetate	1,1-dimethyl-2-phenylethyl acetate	$C_{12}H_{16}O_2$	192.25	0.02973	101/**132**
Allyl cyclohexylpropionate	Allyl-3-cyclohexylpropanoate	$C_{12}H_{20}O_2$	196.29	0.02906	**95**/121
Benzyldimethylcarbinol butyrate	1,1-dimethyl-2-phenylethyl butanoate	$C_{14}H_{20}O_2$	220.31	0.00343	91/**132**
Lilial	3-(4-tert-butylphenyl)-2-methyl-propanal	$C_{14}H_{20}O$	204.31	0.00477	147/**189**
Iso e super	1-(octahydro-2,3,8,8-tetrame-2-naphthalenyl)-1-ethanone	$C_{16}H_{26}O$	234.38	0.00201	109/**191**
Hexyl salicylate	Hexyl-2-hydroxybenzoate	$C_{13}H_{18}O_3$	222.28	0.00003	**138**/222
Yara yara[b]	2-methoxynaphthalene	$C_{11}H_{10}O$	158.20	0.00356	115/**158**
Ethyl salicylate[b]	Ethyl-2-hydroxybenzoate	$C_9H_{10}O_3$	166.17	0.04146	**120**/166

[a] Data obtained from Firmenich's in-house database.

[b] Internal standards

2.2.2 Sample preparation

Two TD tubes (6.0 cm x 0.4 cm id x 0.6 cm od) from Gerstel (Müllheim an der Ruhr, Germany) were each self-packed with 80.0 mg of Tenax® TA and Tenax® GR from Restek Corporation (Bellefonte, USA). Another multisorbent TD tube (6.0 cm x 0.4 cm id x 0.6 cm od) pre-packed with Carbopack™ B, Carbopack™ X and Carboxen™-1000 was purchased from Gerstel (Müllheim an der Ruhr, Germany) for this study. Prior to use, the TD tubes with Tenax® TA and GR were conditioned for 520.00 min at 320°C while the multisorbent tube was conditioned for 520.00 min at 350°C, according to the manufacturer's recommendation. After each analysis, the TD tubes with Tenax® TA and GR were conditioned for 30.00 min at 320°C and 120.00 min at 330°C for the multisorbent tube.

For the selection of sorbent material study, 10.0 mg of perfume A spiked with 0.2 mg yara yara as the internal standard was first dissolved in 1.0 mL of ethanol. One µL of the resulting solution was directly injected into 3 TD tubes each packed with the different sorbents stated above.

For optimisation of the other DHS parameters, unperfumed shampoo base in 10.0-g bulk was first spiked with perfume A at a predetermined dosage of 0.75% and either 1.6 mg of yara yara (for DHS parameters optimisation) or ethyl salicylate (for method validation) and stirred with a glass rod for 3.00 min. The resulting solution was left to macerate for 3.0 h before sampling to ensure that all PRMs had reacted sufficiently with the base. Finally, the solution was weighed into 10 20.0-mL screw cap vials with 1.0 g of sample each and tightly closed using magnetic screw caps with 1.3 mm, 35° Shore A silicone/Polytetrafluoroethylene (PTFE) septa from Gerstel (Müllheim an der Ruhr, Germany).

2.2.3 Instrumentation

An Agilent gas chromatograph 7890B coupled to an Agilent mass selective detector (MSD) 5975C (Santa Clara, California, USA) was used for the GC-MS analysis of all shampoo samples. Sample extraction was carried out at the automated DHS station mounted to a Gerstel Multipurpose Sampler 2 (MPS) coupled to a Gerstel Thermal Desorption Unit (TDU) and a Gerstel CIS 4 programmable temperature vaporisation (PTV) inlet (Müllheim an der Ruhr, Germany), where thermal desorption and cryo-focusing of analytes took place respectively. Data analysis and interpretation were performed on the Gerstel Maestro software (Müllheim an der Ruhr, Germany) integrated with the Agilent GC-MS ChemStation Enhanced Data Analysis (Santa Clara, California, USA). The data acquired were matched against an in-house library, while quantification was performed based on calculated peak areas upon extraction of target ion chromatograms from the Total Ion Chromatogram (TIC). Quantification of the analytes was derived based on internal standard calibration of the samples.

In the sorbent material selection study, the perfume A mixture described in section 2.2.2 was directly injected into the sorbent material, without any DHS sampling being performed. The analytes were then desorbed in the TDU with an initial temperature of 25°C to a final temperature of 250°C for 10.00 min at a ramp rate of 80°C/min. The transfer line between TDU and CIS 4 was constantly heated at 280°C and the desorbed analytes were trapped at -50°C in the CIS 4 baffled, deactivated liner from Gerstel (Müllheim an der Ruhr, Germany) with an equilibration time of 0.20 min. The TDU was cooled with a coolant mixture of ethanol/water in the ratio of 50:50. Following the desorption, the CIS 4 was heated to 250°C at a ramp rate of 12°C/s, and held for 10.00 min. The analytes entered the GC capillary column with a split ratio of 1:10 by setting the helium purge flow to split vent at 13.8 mL/min at 0.00 min.

The GC was equipped with an Agilent 30.0 m x 0.25 mm x 0.25 µm DB-1 MS dimethylsiloxane (Santa Clara, California, USA) capillary column. Helium gas was used as a carrier gas in the constant flow mode at a rate of 1.38 mL/min. The GC oven was programmed as follows: from 50°C ramped at 4°C/min to 85°C, from 85°C ramped at 3.85°C/min to 100°C, from 100°C

ramped at 1.5°C/min to 105°C, from 105°C ramped at 3°C/min to 115°C, from 115°C ramped at 2.7°C/min to 145°C, from 145°C ramped at 2.4°C/min to 165°C, and from 165°C ramped at 30°C/min to 250°C. The temperatures of the transfer line between GC and MS, MS quadrupole and MS electron ionisation source were 280°C, 150°C and 230°C respectively. Solvent delay was set at 3.50 min and the MS was operated in the full-scan mode with a mass range from m/z 29.0 to 400.0.

The analyses of food samples were carried out at Gerstel LLP, Singapore. An Agilent gas chromatograph 7890A coupled to an Agilent MSD 5975 (Santa Clara, California, USA) was used for all GC-MS analyses. The GC was equipped with an Agilent 30.0 m x 0.25 mm x 0.25 µm DB-5 (Santa Clara, California, USA) capillary column. The remaining GC-MS conditions were as described above.

2.2.4 DHS-TD-GC-MS conditions

Where all other DHS parameter optimisations were conducted, each sample was incubated at 30°C and agitated at 500.0 rpm in cycles of 10.00 s followed by 1.00 s without agitation, for 10.00 min. Headspace purging with 600.0 mL of nitrogen gas at a flow rate of 40.0 mL/min was then started while still incubated at 30°C. Target analytes were trapped in a TD tube packed with Tenax® TA at 30°C. The transfer temperature of the DHS needle was kept constant at a maximum temperature of 150°C for direct transfer of VOCs from the sample headspace to the TD tube. Next, the TD tube with adsorbed analytes was dried at 30°C with 840.0 mL of nitrogen gas at a flow rate of 40.0 mL/min to remove any remaining moisture. All samples were analysed in triplicate.

After drying, the loaded TD tube was thermally desorbed in the TDU with helium at a flow of 50.0 mL/min in the solvent venting desorption mode for 2.00 min according to the following heating program: from 30°C with an initial time of 2.00 min, ramped at 80°C/min to 250°C, final hold time for 10.00 min. All other conditions such as the transfer line temperature, CIS 4 temperature program and split ratio remained the same as described in section 2.2.3.

The GC oven was programmed as such: from 50°C ramped at 5°C/min to 90°C, from 90°C ramped at 4°C/min to 100°C, from 100°C ramped at 3°C/min to 105°C, from 105°C ramped at 3.5°C/min to 115°C, from 115°C ramped at 5.5°C/min to 145°C, from 145°C ramped at 5.5°C/min to 165°C, and from 165°C ramped at 30°C/min to 250°C with a final hold time of 5.052 min. All other MS parameters were similar to those stated in section 2.2.3.

While the DHS parameters for the green tea analysis remained the same, the parameters were modified for the milk powder analysis. Each sample was incubated at 60°C and agitated at 500.0 rpm in cycles of 10.00 s followed by 1.00 s without agitation. It was then subsequently purged with 300.0 mL of nitrogen gas at a flow rate of 20.0 mL/min while still being incubated at 60°C. The temperature of the TD tube was set at 40°C to trap analytes. The transfer temperature of the DHS needle was still fixed at 150°C. Lastly, the TD tube with adsorbed analytes was dried at 30°C with 300.0 mL of nitrogen gas at a flow rate of 50.0 mL/min. All samples were analysed in triplicate. The GC oven program was kept the same as stated in the previous paragraph for both food sample analyses.

2.3 Results and discussion

2.3.1 Method optimisation

2.3.1.1 Sorbent material selection

Three different sorbent materials were evaluated to select the best sorbent material through a recovery study: Tenax® TA, Tenax® GR and multisorbent Carbopack™ B, Carbopack™ X and Carboxen™-1000. The recoveries of the PRMs stated in section 2.2.1 were calculated by comparing the peak areas derived from direct liquid injection and after thermal desorption for each sorbent material.[107] The results obtained for Tenax® TA and Tenax® GR are illustrated in **Figure 2.2**, whereas no signal was detected from the multisorbent tube.

For most PRMs, the recoveries recorded for both Tenax® TA and Tenax® GR were relatively similar, except for undecavertol, verdox and

benzyldimethylcarbinol butyrate where their recoveries using Tenax® TA were higher by at least 10.0% than those using Tenax® GR. The relative standard deviations (RSD) for all PRMs in both sorbents were lesser than 10.0%. In greater detail, RSDs ranged from 0.7% (benzyldimethylcarbinol acetate) to 9.8% (limonene) for Tenax® GR, and 0.7% (undecavertol) to 4.5% (zestover) for Tenax® TA, where n=3. Similar trends had been reported in previous volatiles studies where RSDs of volatiles were generally lower in Tenax® TA.[104, 108] Tenax® TA has also been widely adopted in other dynamic headspace studies of wine[95], honey[96], kiwifruit tissue[109], virgin olive oil[110], to name a few. Tenax® TA, a 2,6-diphenylene oxide polymer, is extensively used to trap aroma molecules due to its high affinity for non-polar compounds and low affinity for polar compounds such as water and methanol.[111] Due to its low affinity for water, Tenax® TA is also suitable for trapping VOCs from samples with water content like cooked food.[112] On the other hand, retention of the volatiles could have been too strong on the multisorbent trap, and the volatiles were not desorbed. Similar observations have been reported in other volatile analyses.[108, 113] Therefore, Tenax® TA was chosen as the appropriate sorbent and subsequently used throughout this study.

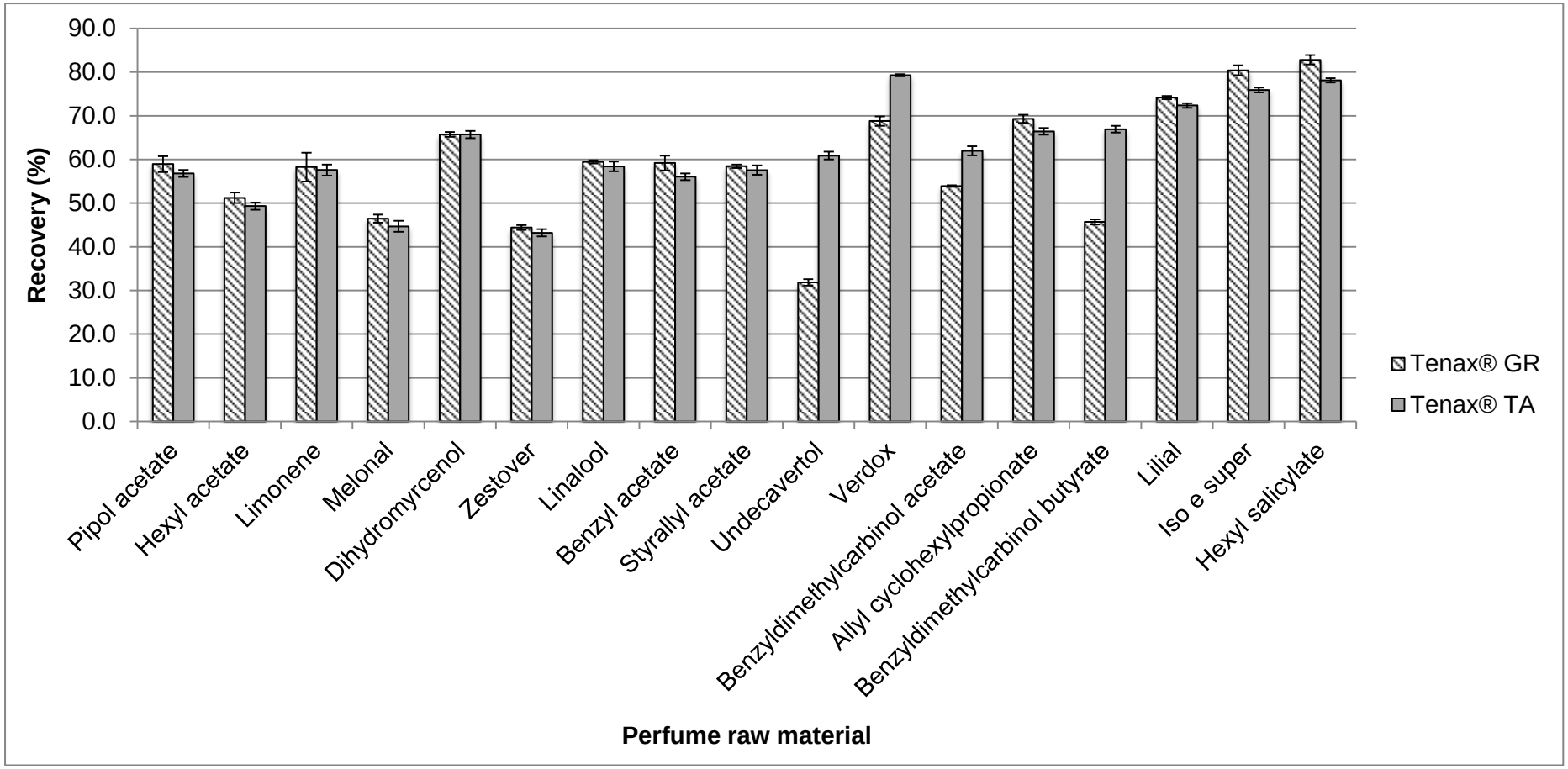

Figure 2.2 Recoveries of perfume raw materials (PRMs) for Tenax® GR and Tenax® TA in triplicate analysis.

2.3.1.2 Incubation temperature

The partition coefficient, K, of an analyte describes its mass distribution in the sample and gas phase, and is defined by the following expression**:**

$$K = \frac{C_S}{C_G} \quad (4)$$

where C_S is the analyte concentration in the sample phase, and C_G is the analyte concentration in the gaseous phase.[114] The partition coefficient is related to temperature, T, as shown:

$$log\, K = \frac{B}{T} - C \quad (5)$$

where B and C are constants specific to the analyte.[114] As T increases, K decreases. The analyte concentration in the gaseous phase increases, indicating the increased amount of analytes absorbed by the sorbent material as volatiles fill the headspace more easily.[115] It would be expected that the analyte concentration in headspace would increase with higher temperatures. However, in this study, the incubation temperature was fixed at 30°C. This could be explained by the occurrence of water condensation in the TD tube at temperatures higher than 40°C, and could not be removed entirely, even with dry purging and solvent venting in the TDU. Presence of water caused ice crystals to form in the CIS during cryo-trapping[108] when the incubation temperature was set at 40°C, 50°C and 60°C, causing the GC inlet to shut down. Not only would this damage the GC, but it would also lead to poor quality results.[108, 116] The RSDs, where n=3, ranged from 1.1% (hexyl acetate) to 12.7% (undecavertol).

2.3.1.3 Incubation time

With the incubation temperature fixed at 30°C, the samples were then incubated in the DHS incubator for 5.00, 10.00, 20.00 and 30.00 min. **Figure 2.3** shows the logarithmic trend of the mean peak areas obtained for all PRMs across the various incubation times. It can be evaluated that dynamic equilibrium had been reached between the sample phase and the gaseous headspace after 10.00 min incubation and agitation. Since equilibrium

remained relatively constant thereafter, an incubation time of 10.00 min was chosen for all experiments subsequently. The RSDs, where n=3, ranged from 1.1% (hexyl acetate) to 12.7% (undecavertol).

2.3.1.4 Trapping phase purge volume

After incubation, the trapping of analytes took place by purging a nitrogen gas flow through the sample and concentrated the analytes on the Tenax® TD tube. It is known that the purge gas volume affects the trapping and the amount of analytes trapped on the sorbent.[95, 104] Previous studies have also reported the increase in analyte recoveries with greater purge volumes, at the same time maintaining at an optimal concentration such that saturation of the adsorbent does not happen.[108, 116] This hypothesis has been proven in the current study. **Figure 2.4** shows the increase in the logarithmic mean peak areas for all PRMs with the increase of the purge volume. However, the purge volume was set at 600.0 mL for subsequent experiments as a compromise between analysis time and extraction efficiency. Excellent RSDs were achieved, ranging from 3.2% (verdox) to 7.1% (lilial), where n=3.

2.3.1.5 Trapping phase purge flow

The other crucial step in the DHS trapping phase is the purge flow.[108] The general trend for the logarithmic mean peak areas of all PRMs as shown in **Figure 2.5** was to reach an optimum of 40.0 mL/min before decreasing steadily at higher purge flow rates. One possible reason for this was the elimination of the analytes with the strong purge flows, even before an equilibrium between the sample phase and the headspace was reached. This observation was also noted by Masuck et al. previously.[108] However, hexyl salicylate was an exception, with its mean peak areas decreasing gradually as the purge flow increased. This could be due to the adsorbent being saturated with PRMs of lower molecular weight and higher volatility. Hexyl salicylate is the heaviest and least volatile among all PRMs. The RSDs, where n=3, ranged from 6.1% (hexyl salicylate) to 11.2% (verdox).

2.3.1.6 Drying phase purge volume

Drying the TD tube filled with analytes after trapping is a common procedure in DHS sampling to remove any moisture content that could enter the GC system.[95, 104, 110, 115] Therefore, the drying phase purge volume has to be optimised to protect the GC-MS systems[95, 104], since they can be damaged by water as explained in section 2.3.1.2. **Figure 2.6** presents the logarithmic trend that all PRMs followed as the volume of nitrogen purging gas flow increased. At 780.0 mL, moisture was still possibly present in the sorbent. The peak areas started to decrease after 840.0 mL, which could be attributed to the excess purging of analytes by nitrogen. Therefore, 840.0 mL was selected as the optimum drying phase purge volume. The RSDs, where n=3, ranged from 1.3% (benzyl acetate) to 11.8% (limonene).

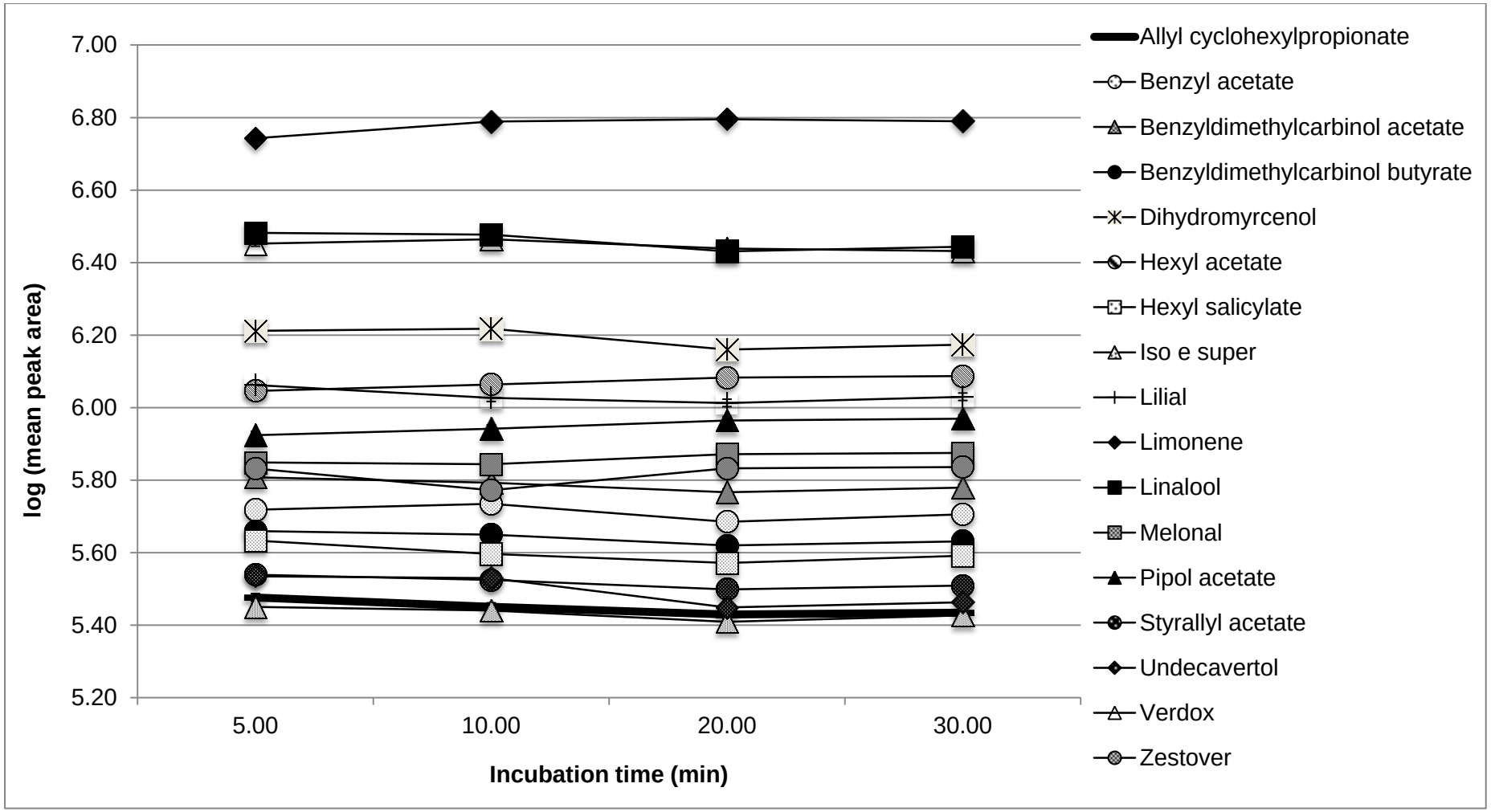

Figure 2.3 Logarithm of mean peak areas of perfume raw materials (PRMs) against incubation time in triplicate analysis.

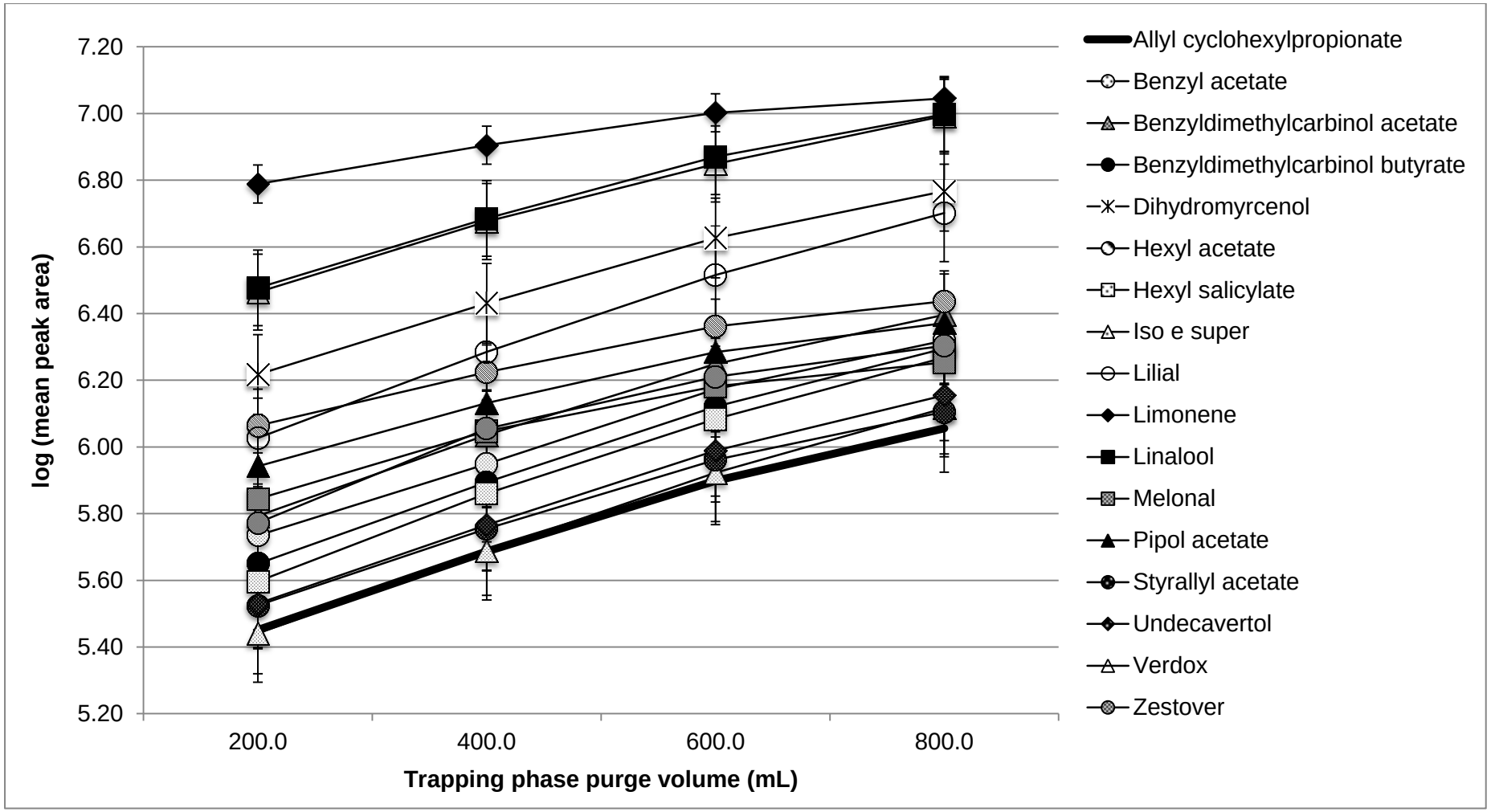

Figure 2.4 Logarithm of mean peak areas of perfume raw materials (PRMs) against trapping phase purge volume in triplicate analysis.

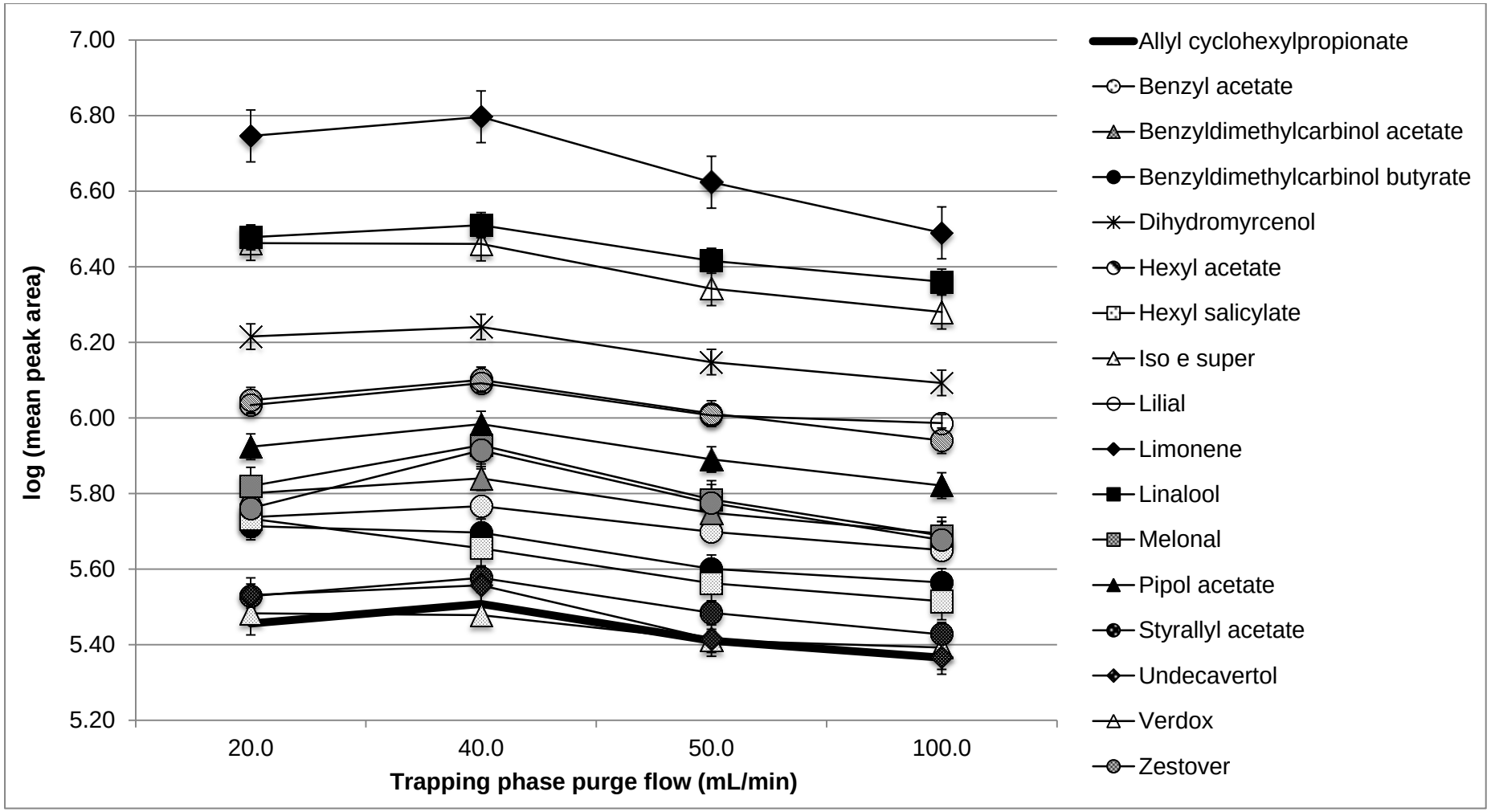

Figure 2.5 Logarithm of mean peak areas of perfume raw materials (PRMs) against trapping phase purge flow in triplicate analysis.

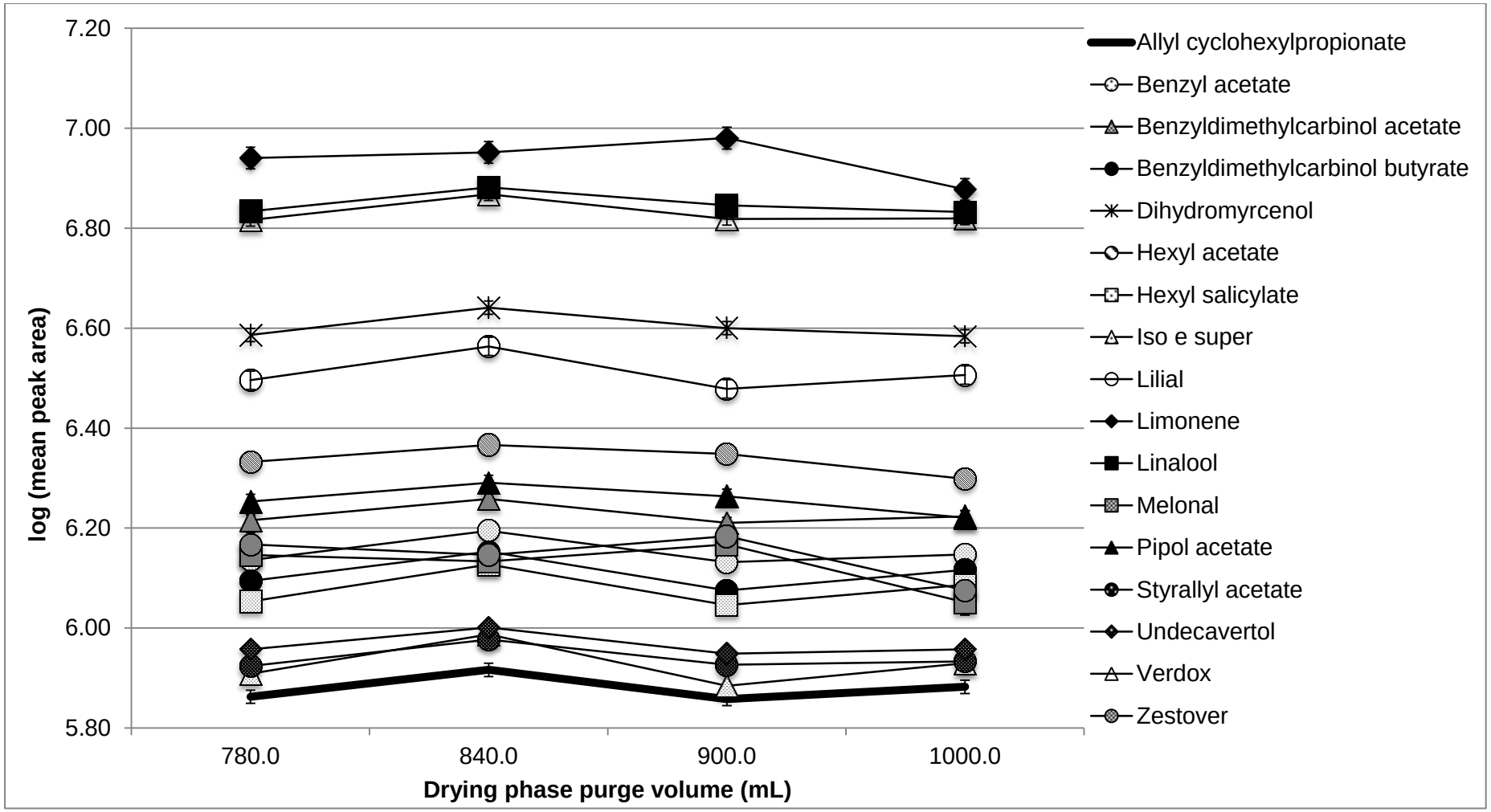

Figure 2.6 Logarithm of mean peak areas of perfume raw materials (PRMs) against drying phase purge volume in triplicate analysis.

2.3.2 Method validation

2.3.2.1 Linearity

The calibration linear range was studied by 2 sets of calibration curves with similar concentration ranges. The concentration range was set using the lowest and highest perfume dosages adopted in industrial applications. This was then applied to quantify the common perfume dosage in shampoos of 0.80% in section 2.3.3.1. The first set of calibration samples were prepared by dosing the unperfumed shampoo base with perfume dosages at 0.02%, 0.06%, 0.24%, 1.01% and 1.21%, and extracted by DHS under the optimised conditions stated in section 2.2.4. Ethyl salicylate (0.16 mg) was added as an internal standard to all samples for quantification. The addition of an internal standard was imperative to attain satisfactory repeatable results[109] and accurate quantitative data for unknown samples.[116] The calibration curves were plotted using the response ratio against concentration ratio. **Figure 2.7** shows the calibration curve for set 1, with an overall coefficient of determination (R^2) of 0.9997. Following that, the calibration curve was duplicated on a different day. The unperfumed shampoo base samples were spiked at 0.02%, 0.06%, 0.26%, 1.04% and 1.24% of perfume A. Similarly, they were extracted by DHS under the optimised conditions stated in section 2.2.4. The set 2 calibration curve is shown in **Figure 2.8**, with an overall R^2 of 0.9989. **Table 2.2** presents a good linearity obtained in both studies over 2 different days with the individual R^2 of all analytes not lesser than 0.9835.

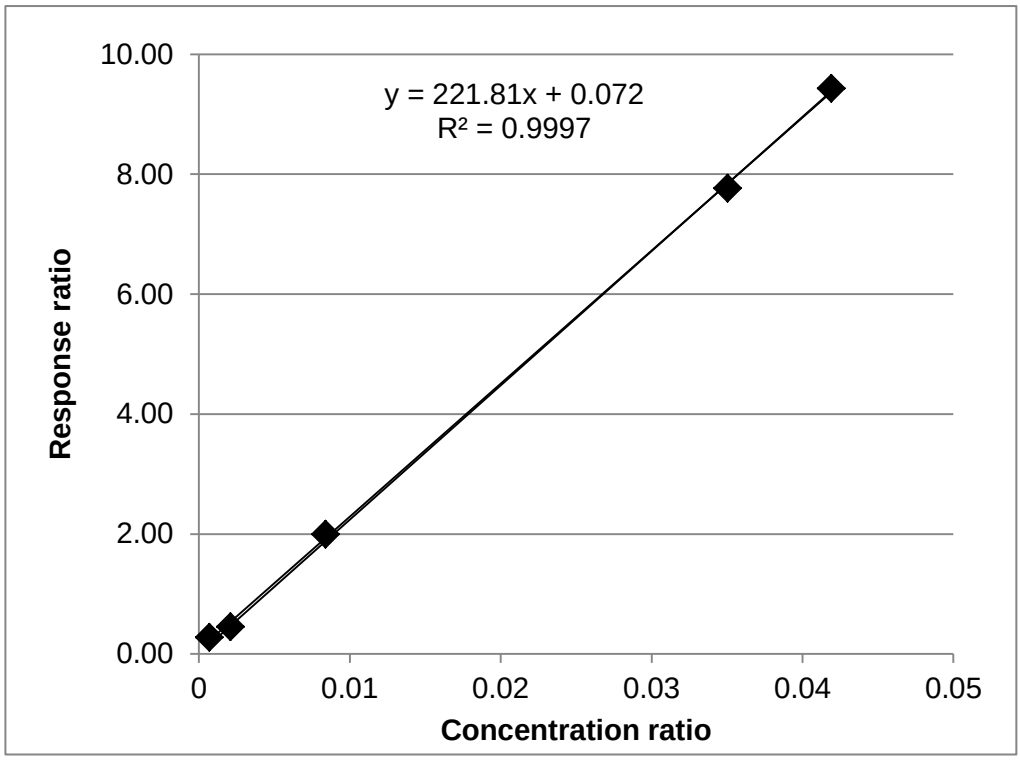

Figure 2.7 Set 1 calibration curve of 5 shampoo samples spiked with perfume dosages of 0.02%, 0.06%, 0.24%, 1.01% and 1.21%.

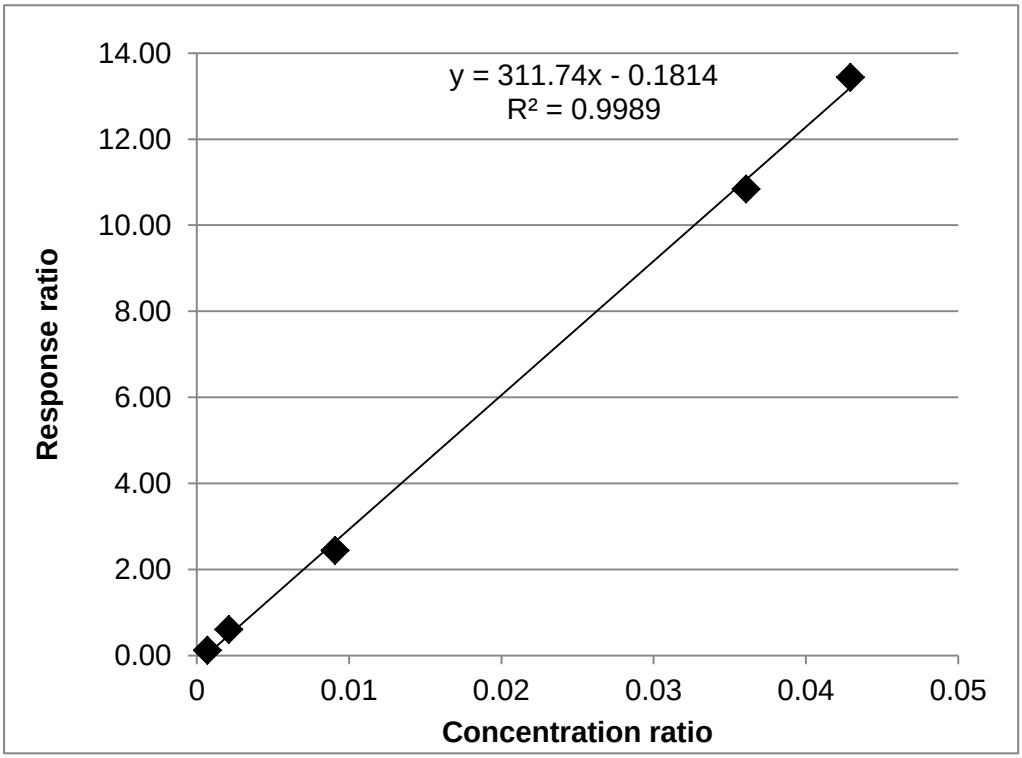

Figure 2.8 Set 2 calibration curve of 5 shampoo samples spiked with perfume dosages of 0.02%, 0.06%, 0.26%, 1.04% and 1.24%.

Table 2.2 Calibration linearity for all perfume raw materials (PRMs) derived from 2 calibration sets performed on 2 different days.

PRM	R^2	
	Set 1 (perfume dosage: 0.02% to 1.21%)	Set 2 (perfume dosage: 0.02% to 1.24%)
Pipol acetate	0.9945	0.9835
Hexyl acetate	0.9971	0.9933
Limonene	0.9962	0.9985
Melonal	0.9987	0.9958
Dihydromyrcenol	0.9985	0.9965
Zestover	0.9993	0.9994
Linalool	0.9985	0.9993
Benzyl acetate	0.9956	0.9865
Styrallyl acetate	0.9960	0.9876
Undecavertol	0.9957	0.9929
Verdox	0.9985	0.9968
Benzyldimethylcarbinol acetate	0.9967	0.9976
Allyl cyclohexylpropionate	0.9969	0.9944
Benzyldimethylcarbinol butyrate	0.9969	0.9956
Lilial	0.9997	0.9960
Iso e super	0.9992	0.9958
Hexyl salicylate	0.9961	0.9932

2.3.2.2 Repeatability

Both intra-day and inter-day repeatability studies were performed to establish the method's precision. For the intra-day repeatability study, 5 identical unperfumed shampoo base samples of 1.0 g each were dosed with perfume A at 0.75% dosage and 0.16 mg of yara yara as the internal standard. The samples were extracted by DHS under the optimised conditions stated in section 2.2.4 within the same day. Good RSDs were achieved for all PRMs, with the highest at 10.4%. For the inter-day repeatability study, 9 identical unperfumed shampoo base samples of 1.0 g were dosed with perfume A at 0.75% dosage and 0.16 mg of yara yara as the

internal standard. Three samples were extracted by DHS under the optimised conditions stated in section 2.2.4 on each day for 3 days. Satisfactory RSDs were achieved for all PRMs, with the highest at 15.9%. However, the RSDs of pipol acetate, hexyl acetate, limonene and melonal fluctuated across the 3 days. This was likely due to the high volatility nature of these molecules, in addition to their low amounts in the perfume. All results for the repeatability studies are presented in **Table 2.3**.

2.3.2.3 Sensitivity

The sensitivity of the method was demonstrated by the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ of all PRMs were estimated by a signal-to-noise ratio (S/N) of 3 and 10 respectively, using the calibration sample spiked with 0.02% perfume A. The lowest LOD and LOQ deduced were found to be that of hexyl acetate, at 3.6 μg g^{-1} and 11.9 μg g^{-1} respectively. However, the LOD and LOQ for benzyldimethylcarbinol acetate and hexyl salicylate could not be determined due to co-elution with the minor isomer of verdox and iso e super respectively. The LOD and LOQ of all PRMs are shown in **Table 2.3**.

2.3.3 Method application

2.3.3.1 Accuracy in quantification of perfume in shampoo

To assess the applicability of the DHS method for fragrance consumer products, 1.0 g of unperfumed shampoo base was spiked with perfume A at 0.801% and 0.179% dosages separately, and 0.16 mg of ethyl salicylate as the internal standard. Three samples of each dosage were extracted by DHS under the optimised conditions stated in section 2.2.4. The samples of the 2 different dosages were calibrated and quantified by calibration curves 1 and 2 respectively. Both sets of mean experimental dosages were close to the target dosages spiked; the mean dosage of the first set of samples was found to be 0.787% against the target dosage of 0.801%. In addition, the mean dosage of the second set of samples was deduced to be 0.181% in contrast to the target dosage of 0.179%.

Table 2.3 Precision of perfume raw materials (PRMs) in 0.75% dosage. Limits of detection and quantification determined by 0.02% dosage.

PRM	Intra-day precision (%RSD, n=5)	Inter-day precision (%RSD, n=3)			LOD (μg g^{-1})	LOQ (μg g^{-1})
		Day 1	Day 2	Day 3		
Pipol acetate	5.79	3.34	11.65	4.56	5.1	17.0
Hexyl acetate	6.25	2.04	12.27	2.96	3.6	11.9
Limonene	10.44	3.10	15.86	2.51	134.7	448.8
Melonal	5.70	1.85	10.50	3.37	5.9	19.8
Dihydromyrcenol	3.47	1.80	4.35	3.83	12.3	41.1
Zestover	4.58	1.02	6.94	3.87	4.6	15.3
Linalool	4.05	0.59	3.77	3.63	10.5	35.1
Benzyl acetate	3.67	0.48	1.82	5.82	6.3	21.1
Styrallyl acetate	4.24	1.20	1.98	4.41	5.6	18.8
Undecavertol	3.46	4.61	0.52	7.01	36.7	122.5
Verdox	3.15	1.24	6.55	3.99	10.1	33.7
Benzyldimethylcarbinol acetate	3.88	0.43	1.89	4.91	-	-
Allyl cyclohexylpropionate	3.94	0.91	1.42	5.42	13.6	45.4
Benzyldimethylcarbinol butyrate	2.90	1.64	1.01	6.37	22.1	73.6
Lilial	1.52	0.41	2.50	7.81	62.4	208.0
Iso e super	2.27	1.21	3.12	4.41	92.4	307.9
Hexyl salicylate	1.16	0.40	0.57	7.65	-	-

2.3.3.2 Analysis of green tea

The DHS methodology was further applied to selected common food products. One gram of green tea was weighed into a 20.0-mL headspace vial, and spiked with 10.0 ng of styrallyl acetate as the internal standard. The internal standard solution was prepared through a serial dilution with methanol. Three spiked green tea samples were extracted by the optimised conditions stated in section 2.2.4. The samples were analysed in the GC using the splitless mode while the oven program was kept as described in section 2.2.4. The quantified mass of each target analyte in the sample was estimated by a comparison between its GC peak area and that of the internal standard, assuming a response factor of 1, as adopted previously by Lignou et al.[117] This can be represented by the following equation:

$$m_a = \frac{A_a}{A_{istd}} \times \frac{m_{istd}}{1} \quad (6)$$

where:

A_a is the mean peak area of the analyte from the chromatogram

A_{istd} is the peak area of the internal standard from the chromatogram

m_a is the mass of the analyte in the sample

m_{istd} is the known mass of the internal standard added

Ten compounds were identified in each green tea sample. The peak area of each analyte in each sample, the mean peak area of each analyte in 3 samples, the RSD as well as the mean quantified mass of each analyte are shown in **Table 2.4**. The RSDs, where n=3, ranged from 2.3% (limonene) to 13.7% (jasmone). Based on previous studies on volatiles analysis of jasmine tea, linalool, benzyl acetate, methyl benzoate, methyl salicylate and methyl anthranilate were significant of jasmine aroma.[118-119] In a study conducted by Ito et al.[118], the floral note of linalool, a common aroma active compound present in plants, was identified through GC-O. Other aroma characteristics identified were the floral note of benzyl acetate, the floral and green notes of methyl salicylate, and the citrusy, fruity notes of methyl anthranilate. Methyl anthranilate was also noted to possess a sweet, grape-like smell that is characteristic of the *Jasminum sambac* flower species.[120] In another study on the odour contribution by various volatiles in different grades of green tea, D-limonene and linalool were reported to be harsh, raw and sharp. Additionally,

linalool also possessed sweet, floral and fruity notes.[121] A sample chromatogram of one of the green tea sample is shown in **Figure 2.9**.

Table 2.4 Peak areas, mean peak areas, % RSD and mean quantified mass of all analytes in 3 different green tea samples.

Analyte	Peak area 1	Peak area 2	Peak area 3	Mean peak area	%RSD	Mean mass (ng)
Methyl butanoate	114996	95986	99722	103568	7.94	6.8
Hexenyl pentanoate	30331	35895	31448	32558	7.38	2.1
2-ethyl-hexanol	55094	49343	46877	50438	6.83	3.3
Limonene	22272	21155	21340	21589	2.26	1.4
Methyl benzoate	80364	102934	93997	92431.67	10.04	6.1
Linalool	841156	718634	610526	723438.67	13.02	47.4
Benzyl acetate	3468548	4042209	4732904	4081220.33	12.67	267.3
Methyl salicylate	82821	88627	79006	83484.67	4.74	5.5
Methyl anthranilate	26221	33618	36279	32039.33	13.28	2.1
Jasmone	3540	4540	4957	4345.67	13.68	0.3
Styrallyl acetate*	173121	132251	152692	152688	10.93	10.0

*Internal standard

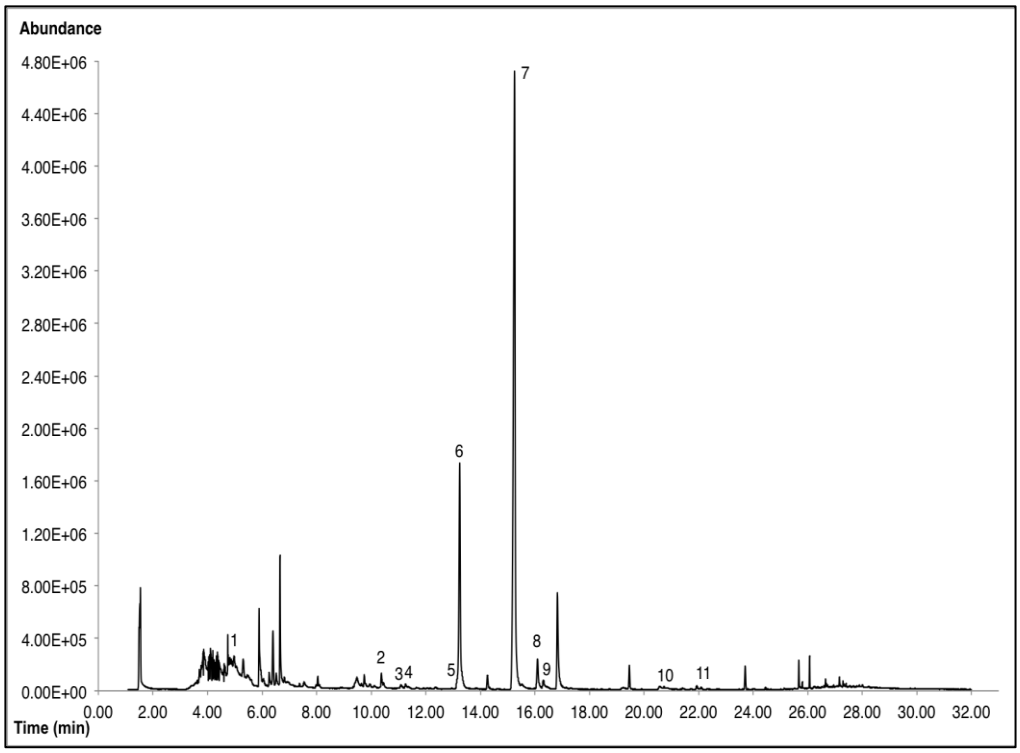

Figure 2.9 Gas chromatography-mass spectrometry (GC-MS) chromatogram of a green tea sample.

Labeled peaks: (1) Methyl butanoate; (2) Hexenyl pentanoate; (3) 2-ethylhexanol; (4) Limonene; (5) Methyl benzoate; (6) Linalool; (7) Benzyl acetate; (8) Styrallyl acetate (internal standard); (9) Methyl salicylate; (10) Methyl anthranilate; (11) Jasmone

2.3.3.3 Analysis of milk flavour

In addition to green tea, the DHS methodology was also employed for milk flavour analysis. Similar to the green tea analysis, 1.0 g of milk flavour was weighed into a 20.0-mL headspace vial, and spiked with 10.0 ng of styrallyl acetate as the internal standard. Three spiked milk flavour samples were extracted by the optimised conditions stated in section 2.2.4. Milk products are noted to have lower amounts of flavour generating compounds as compared to other food products[122], hence the need to increase the incubation temperature in this study in order to intensify the amount of volatiles entering the sample headspace. It has been reported previously that heating skim milk powder and dairy products led to increased intensity and recovery of their volatile flavour compounds.[123-124] However, 60°C was selected as the incubation temperature here as thermal reactions could take place in milk products at temperatures higher than that.[122] 60°C was also

sufficient to obtain satisfactory analyte peak responses. The samples were analysed in the GC using a split ratio of 20:1 while the oven program was kept as described in section 2.2.4. The quantified mass of each target analyte in the sample was also estimated by a comparison between its GC peak area and that of the internal standard, assuming a response factor of 1. Twelve compounds were identified in each milk flavour sample. The peak areas of each analyte in each sample, mean peak areas of each analyte in 3 samples, RSDs where n=3 as well as the mean quantified mass of each analyte are presented in **Table 2.5**. The RSDs obtained ranged from 3.3% (butyl butyryl lactate) to 16.1% (vanillin). Some odour attributes of skim milk flavour have previously been reported. Generally, lactones, together with fatty acids, contribute to the distinctive flavour of skim milk powder. Shiratsuchi et al[125] noted the sweet, milky odour contributed by gamma-undecalactone, while delta-decalactone had sweet, fatty and milky odour characteristics. A sample chromatogram of one milk flavour sample is shown in **Figure 2.10**.

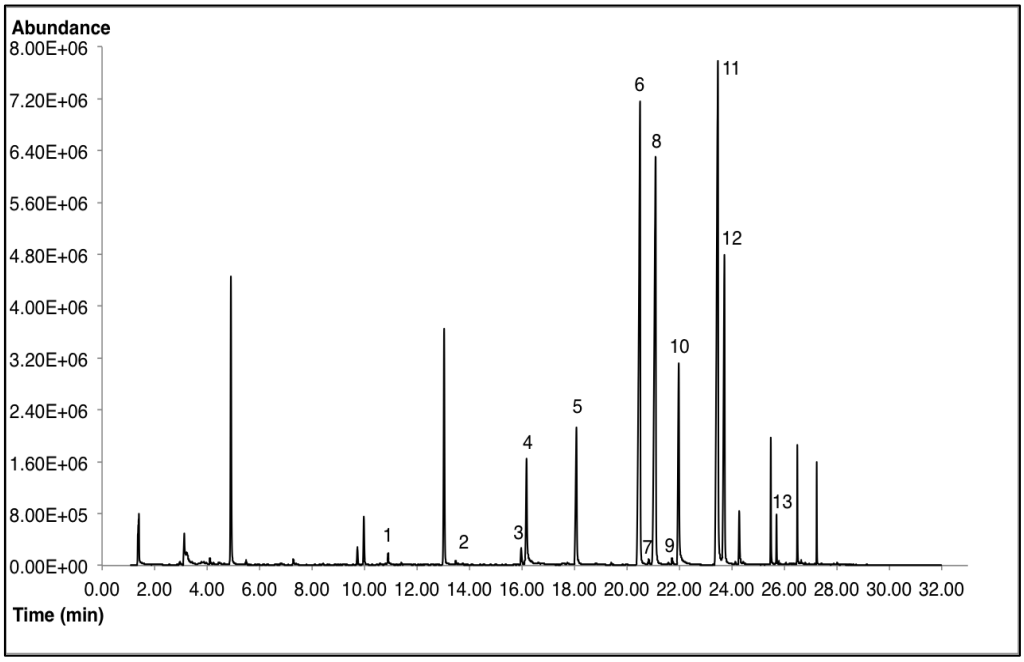

Figure 2.10 Gas chromatography-mass spectrometry (GC-MS) chromatogram of a milk flavour sample.

Labeled peaks: (1) Limonene; (2) p-Cymene; (3) Styrallyl acetate (internal standard); (4) Ethylmaltol; (5) Gamma-nonalactone; (6) Triacetin; (7) Butyl butyryl lactate; (8) Gamma-decalactone; (9) Delta-decalactone; (10) Vanillin; (11) Ethyl vanillin; (12) Gamma-undecalactone; (13) Ethyl laurate

Table 2.5 Peak areas, mean peak areas, %RSD and mean quantified mass of all analytes in 3 different milk flavour samples.

Analyte	Peak area 1	Peak area 2	Peak area 3	Mean peak area	%RSD	Mean mass (ng)
Limonene	56209	54682	60957	57282.67	4.66	4.3
p-Cymene	22417	24662	25882	24320.33	5.90	1.8
Ethyl maltol	1543014	1331339	1262938	1379097	8.65	103.4
Gamma-nonalactone	3414552	3852792	3553406	3606916.67	5.07	270.5
Triacetin	2812518	3507514	3148699	3156243.67	8.99	236.7
Butyl butyryl lactate	93917	97081	89591	93529.67	3.28	7.0
Gamma-decalactone	11613227	14242367	12898344	12917979.33	8.31	968.6
Delta-decalactone	60400	70966	62359	64575	7.11	4.8
Vanillin	1718325	2569023	2285308	2190885.33	16.14	164.3
Ethyl vanillin	12227727	12449586	10077884	11585065.67	9.23	868.7
Gamma-undecalactone	6088088	6931394	6068269	6362583.667	6.32	477.1
Ethyl laurate	194238	207379	192444	198020.33	3.36	14.8
Styrallyl acetate*	147885	136682	115532	133366.33	10.06	10.0

* Internal standard

2.4 Conclusion

A fully automated DHS-TD-GC-MS method was developed in this study for the quantification of perfume and food VOCs. The main parameters optimised were the incubation temperature, incubation time, trapping phase purge volume and flow, as well as the drying phase purge volume. This solvent-less technique was further validated and displayed good linearity in the target concentration range. Satisfactory repeatability and sensitivity were also achieved as demonstrated by the RSDs as well as LOD and LOQ respectively. Finally, the method was reliably applied to analysis of flavour and fragrance consumer products, and proved its merit over other extraction techniques in volatiles analysis across different sample matrices.

Chapter 3. Method development for the quantification of fragrance volatiles on hair deposition

3.1 Introduction

Fragrance has many applications in a wide variety of consumer products today, including fine fragrances, home care, body care and hair care products. Generally consumer products such as personal care and household products contain functional fragrances, which are created through the blending of a myriad of aroma chemicals, ranging from 0.01% to 100% dosage depending on the product.[126] A perfume is evaluated traditionally through trials and its applied products, but this approach has gradually shifted as more focus has been placed on the capability of individual PRMs in applied products recently. This would benefit both the perfumer and the consumer to make sure that the created perfume is remarkable for the former, and to benefit from the perfume design such as its ability to improve the product's performance for the latter.[25]

However, the cleansing role of cosmetic products is not played by a perfume. Instead, it is vital for enticing consumers by masking any malodours and to retain the fragrance for the longest time possible.[127] In soap perfumery, perfumed shampoo stands out from other cosmetic soap products. It plays a significant role in imparting an aroma to hair as an integral part of one's personal odour, where the fragrance is first absorbed by the hair before dispersing. Today, the use for after-treatments of hair is no longer mandatory. Therefore, the skilful selection of perfume to be added to a shampoo is required. The fragrances ought to be strong, of decent quality and be able to blend well with the hair's natural odour. For example, some PRMs that carry with them the unique sweet-nutty note of lactones are especially appropriate as it is also inherent of hair odour.[128] Other than the basic function of cleaning, variants have appeared for anti-dandruff and hair health promotion. Therefore, it must be ensured the perfumes do not react with such additives yet maintaining performance.[126]

3.1.1 Perfume substantivity

The performance of a perfume can be assessed by different factors such as its long-lastingness. As explained in Chapter 1, perfume substantivity is one property that determines the longevity of a perfume through its molecular interactions with substrates like fabric, hair and skin.[30] The definition of perfume substantivity encompasses both the bonding between a perfume and a substrate, and also delivery barriers.[29] These barriers refer to the transfer of perfume from certain products like soap through a medium such as an aqueous solution to the substrate, as compared to a cologne where direct application on the substrate is possible and powerful.[29] This could possibly be explained by the octanol-water partition coefficient, *log* P, since it represents the chemical's solubility, thereby deciding the PRMs partitioning in the substrate.[29] Also, *log* P has been proven to increase linearly with the logarithm of the partition coefficient between an odorant in the aqueous fabric softener and the fabric substrate itself.[129-130] Thus, it is not difficult to understand why components with higher *log* P tend to migrate to the hydrophobic substrate surface.

3.1.2 Previous studies on perfume substantivity

A well-performing perfume is diffusive and substantive.[131] Research on perfume deposition and release on substrates remains pivotal in the fragrance industry,[132] in order for the continuous development of impressive perfumes. One of which is PRMs in detergents and softeners and its applications on fabric. A study, based on fractional factorial design, was conducted by Escher and Oliveros[129] to identify some factors that could impact the perfume substantivity on laundered and dried fabrics. Utilising liquid scintillation counting (LSC), they concluded that the fabric type and the nature of surfactants found in detergents mainly affected the substantivity during laundering and drying-out.[129]

Some factors in other studies which were also noted to affect substantivity are the odorant's vapour pressure, water solubility, odour threshold, specific functional groups as well as matrix effect displayed by the fabric.[131] When terry cloth was washed with a fabric softener, the results

observed were in line with its sensory data. The more volatile materials, benzyl acetate, linalool and α-ionone, were in higher amounts in the wet stage with the exception of benzyl acetate due to its high odour threshold, thus attaining a low odour activity value. A molecule's odour threshold value is defined as the lowest concentration that it can provide an odour impression.[133] As these materials undergo drying, they were lost readily due to their high evaporation rate, and not detected much in dry laundry. On the other hand, even at low concentrations, moderate to low volatile materials were found to be rather substantive due to their lower odour thresholds. In summary, ingredients with high odour activity values and low odour thresholds are favourable for high perfume substantivity on dry fabric.[131]

One technique that has been applied in measuring a PRM, galaxolide®, on fabric is the direct analysis in real time (DART)-MS. The signal intensities obtained reflected an approximate linear correlation to the sample amount at different dosages. At various dry stages, the signal intensities did not have much change, which was expected as galaxolide® is a low volatile musk. The experiment was further applied to galaxolide® deposition on hair, and it was identified even on single hair. Even though DART is a rapid and sensitive technique, it is very much dependent on the sample amount exposed to the ionisation area, thus involving specificity of the sample shapes and manner that the samples are exposed to the ion source.[132] Also, some other limitations of DART are its inability to distinguish isomers and stereoisomers, lower sensitivity compared to GC-MS, no structural information obtained, unable to provide quantitative data for manual sample introduction. Lastly, the complex mass spectra require additional interpretation, especially for samples with many compounds.[134]

Fluorescence microscopy is another technique that has been explored in fragrance deposition studies. Fluorescent dyes were added into perfume oil and also attached to the perfume microcapsules, for the measurement and quantification of these microcapsules deposited on fabric through image analysis.[135] Direct measurements of silicone deposits on hair were also investigated using X-ray fluorescence (XRF) by image analysis.[136-139] Despite being non-destructive, the disadvantage of XRF in this scenario is that it is specific to only silicone chemicals, yet being unable to discriminate the sources of the silicone materials, whether they are from the hair strand itself

or the applied products.[140] Furthermore, XRF is used mostly as a qualitative technique in such studies because only silicone deposited on hair surface is measured, since hair conditioning merely affects the surface instead of the hair bulk. Therefore, when an X-ray beam is shone on hair fibres, only atoms on the surface hair fibres are excited and emit fluorescence at particular energy levels, once they return to the ground state. Finally, another limitation is consistent results are only obtained if XRF is performed on a single direction of hair arrangement.[138]

There are also a few deposition studies that employed the indirect measurement approach. This is commonly achieved by extracting the hair sample, which has been washed using an applied product, with an organic solvent. From which, the extract is analysed to find out its contents of ingredients. Various organic solvents are used depending on the polarity of the target analyte, such as toluene, methyl isobutyl ketone, methanol, trichloromethane or chloroform.[140-141] Other than environmental and health concerns associated with the use of organic solvents as discussed in Chapter 2, another concern of using solvent extraction is that the extraction may not be complete if PRMs are too strongly attracted to hair. This is further complicated by the fact that the extraction efficiency of hair is not known.

In view of these reasons, Huang et al.[140] developed an improvised method by determining the amount of ingredients in the water used to rinse off the applied product. Then, the deposited amount can be derived from deducting the rinsed portion from the original applied amount.[140] Although this method does not make use of any organic solvents and the hair samples can be re-used, it is not a direct measurement and quantification of the deposited PRMs. Also, it is based on the assumption that the total amount of PRMs is either transferred to the rinse water or deposited on hair. Other possibilities such as the vaporisation of the PRMs during application and washing of the hair are not accounted for. Therefore, the quantification of PRMs for a deposition study is only accurate if it was a direct measurement from the hair swatch itself.

3.1.3 Objective

Some consumers are particular about the performance of a shampoo's fragrance, as this is where they develop a liking for hair washing. Research has shown that there was increased consumer preference for substantive fragrance in hair care products for two main reasons: people pay attention to hair smells and the desire to remove environmental odours which can lead to undesirable smell on hair. For these reasons, fragrance impacts heavily on the selection of a particular shampoo and ultimately recognition of its brand.[142] Since fragrance deposition, particularly in the area of direct measurement of chemicals on substrates, has not been widely studied, a new non-destructive method for the direct measurement of PRMs deposition on hair is presented in this study.

The building of knowledge of the affinities of PRMs with hair aids perfumers and fragrance development personnel in achieving and developing superior yet cost effective products.[140] A further development of the automated DHS-TD-GC-MS, the automated Large Volume Dynamic Headspace-Thermal Desorption-Gas Chromatography-Mass Spectrometry (LVDHS-TD-GC-MS) was used for the PRMs quantification. The working principle of LVDHS-TD-GC-MS is identical to that of the automated DHS-TD-GC-MS described in Chapter 2. However, instead of analysing the neat sample, an applied sample, the hair swatch in this case, was analysed directly (**Figure 3.1**).

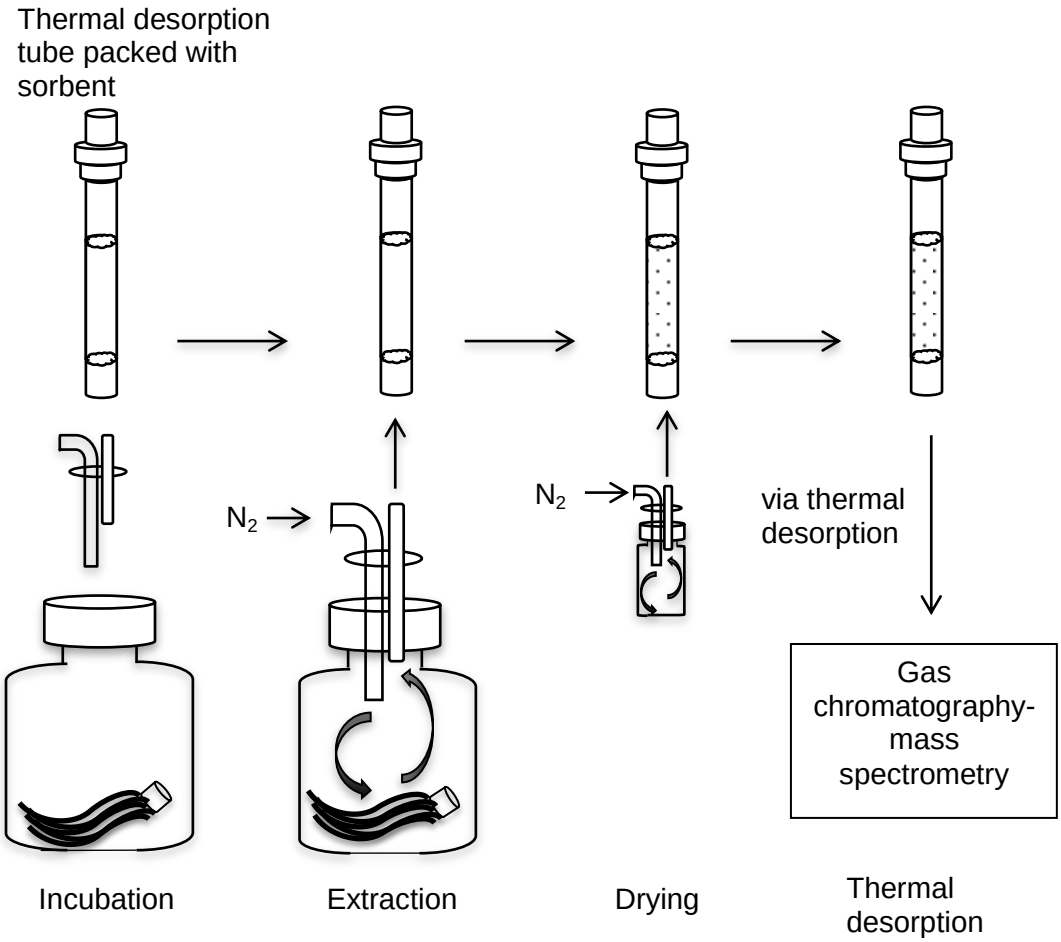

Figure 3.1 Schematic diagram of large volume dynamic headspace (LVDHS) sampling.

Similar DHS parameters such as incubation temperature, incubation time, extraction volume, extraction flow and drying volume were optimised. This method was then validated and employed for the quantitative analysis of rinsed-off hair swatches.

3.2 Experimental materials and methods

3.2.1 Chemicals and reagents

Perfume B was an in-house perfume that contained the following PRMs: dihydromyrcenol (2.1% w/w), linalool (8.5% w/w), benzyl acetate (0.5% w/w), styrallyl acetate (0.4% w/w), verdox (4.2% w/w), benzyldimethylcarbinol acetate (1.1% w/w), allyl cyclohexylpropionate (1.6% w/w), benzyldimethylcarbinol butyrate (2.3% w/w), lilial (9.2% w/w), iso e super (6.7% w/w) and hexyl salicylate (12.0% w/w). The chemical names, chemical formulae, molecular weights, *log* P and selected ions for quantification can be referred to in **Table 3.1**. The unperfumed pearly shampoo base (Base no.: EDL-13-001) was an in-house base. Reagent alcohol, methanol and isopropyl alcohol were purchased from Tritech Scientific Pte. Ltd, Singapore. Sodium laureth sulfate was an in-house surfactant.

Table 3.1 Chemical names, chemical formulae, molecular weights, *log* P and quantifying ions for all perfume raw materials (PRMs). The target ions have been marked in bold.

PRM	Chemical name[a]	Chemical formula[a]	Molecular weight (g mol^{-1})[a]	*log* P[a]	Quantifying ions (m/z)
Dihydromyrcenol	2,6-dimethyl-7-octen-2-ol	$C_{10}H_{20}O$	156.27	3.21	**59**/123
Linalool	3,7-dimethyl-1,6-octadien-3-ol	$C_{10}H_{18}O$	154.25	2.94	**71**/93
Benzyl acetate	Benzyl acetate	$C_9H_{10}O_2$	150.17	2.04	91/**108**
Styrallyl acetate	1-phenylethyl acetate	$C_{10}H_{12}O_2$	164.20	2.22	**104**/122
Verdox	2-tert-butylcyclohexyl acetate	$C_{12}H_{22}O_2$	198.30	4.40	**82**/123
Benzyldimethylcarbinol acetate	1,1-dimethyl-2-phenylethyl acetate	$C_{12}H_{16}O_2$	192.25	3.45	101/**132**
Allyl cyclohexylpropionate	Allyl-3-cyclohexylpropanoate	$C_{12}H_{20}O_2$	196.29	4.51	**95**/121
Benzyldimethylcarbinol butyrate	1,1-dimethyl-2-phenylethyl butanoate	$C_{14}H_{20}O_2$	220.31	4.42	91/**132**
Lilial	3-(4-tert-butylphenyl)-2-methyl-propanal	$C_{14}H_{20}O$	204.31	3.90	147/**189**
Iso e super	1-(octahydro-2,3,8,8-tetrame-2-naphthalenyl)-1-ethanone	$C_{16}H_{26}O$	234.38	5.24	109/**191**
Hexyl salicylate	Hexyl-2-hydroxybenzoate	$C_{13}H_{18}O_3$	222.28	5.55	**138**/222

[a] Data obtained from Firmenich's in-house database.

3.2.2 Sample preparation

Ten TD tubes (6.0 cm x 0.4 cm id x 0.6 cm od) from Gerstel (Müllheim an der Ruhr, Germany) were each self-packed with 80.0 mg of Tenax® TA from Restek Corporation (Bellefonte, USA). Conditionings of the TD tubes were as described in section 2.2.2.

For optimisation of the LVDHS parameters, 5.0 g of unperfumed shampoo base was spiked with perfume B at a predetermined dosage of 0.008% and stirred with a glass rod for 3.00 min. The resulting solution was left to macerate for 3.0 h before using to ensure that all PRMs had reacted sufficiently with the base. Virgin black hair swatches from Indonesia [1], measured approximately 15.5 cm in length, 5.5 cm in width and 25.0 g in weight, were used in this experiment. For each analysis, 1 hair swatch was dampened under water with a flow rate of about 2.0 L/min at 37°C for 30.00 s. A portion of the prepared shampoo sample (2.5 g) was first applied on the hair swatch and foamed for 30.00 s. The hair swatch was then rinsed under the tap with a flow rate of about 2.0 L/min at 37°C for 30.00 s. The remaining 2.5 g of the prepared shampoo sample was subsequently applied on the hair swatch and again rubbed for 30.00 s. Following that, the hair swatch was again rinsed under the tap with a flow rate of about 2.0 L/min at 37°C for 30.00 s. The hair swatch was wrung 10 times before leaving it to dry on a holder for 24.0 h.

After drying for 24.0 h, the hair swatch was placed in a 650.0-mL sample vessel and tightly secured with a Viton® sealing ring between the vessel and its cover. The cover was tightly sealed with a 1.3-mm, 35° Shore A silicone/PTFE septum. All consumables including the sample vessel with cover, sealing ring and the septa were purchased from Gerstel (Müllheim an der Ruhr, Germany). For method validation, samples were placed in 500.0-mL sample vessels that were used together as a set with the DHS^{large} autosampler from Gerstel (Müllheim an der Ruhr, Germany), to analyse samples in series.

At the end of each analysis, the hair swatch was decontaminated before re-using for the next analysis. The hair swatch was first soaked in

[1] Unable to state company name due to confidentiality

about 170.0 mL of 4.00% sodium laureth sulfate/water solution for 4.0 h, rinsed and left to dry. Once dried, the hair swatch was then soaked in about 340.0 mL of 2.00% isopropyl alcohol/water mixture for 2.0 h, rinsed and stand to dry. All hair swatches were ensured to not have any odour or fragrance before use.

3.2.3 Instrumentation

An Agilent gas chromatograph 7890B coupled to an Agilent MSD 5975C (Santa Clara, California, USA) was used for the GC-MS analysis. Sample extraction was carried out at the automated DHS^{large} station from Gerstel (Müllheim an der Ruhr, Germany) mounted to a Gerstel MPS 2 (Müllheim an der Ruhr, Germany), where only 1 sample could be extracted at each time. For method validation, sample extraction was conducted using the DHS^{large} autosampler from Gerstel (Müllheim an der Ruhr, Germany) to analyse a series of samples in the same sequence. The GC configurations, MS conditions and data analysis were kept the same as described in section 2.2.3. Quantification of the analytes was derived based on external calibration of the samples.

3.2.4 LVDHS-TD-GC-MS conditions

Each hair swatch was incubated at 30°C for 1.0 h without agitation. Headspace purging with 3000.0 mL of nitrogen gas at a flow rate of 100.0 mL/min was then started while still being incubated at 30°C. Target analytes were trapped in a TD tube packed with Tenax® TA at 30°C. The transfer temperature of the DHS needle was kept constant at a maximum temperature of 150°C for direct transfer of VOCs from the sample headspace to the TD tube. Next, the TD tube with adsorbed analytes was dried at 40°C with 500.0 mL of nitrogen gas at a flow rate of 100.0 mL/min to remove any water vapour. All samples were analysed in triplicates.

The TDU and CIS 4 conditions remained the same as described in section 2.2.4. The analytes entered the GC capillary column in a splitless

mode by setting the helium purge flow to split vent at 13.8 mL/min at 2.00 min for parameters optimisation to enhance the signal intensities, since the perfume dosage in the shampoo samples used to rinse the hair swatch was very low. However, during method validation, a GC split ratio of 10:1 was used by setting the helium purge flow to split vent at 13.8 mL/min at 0.00 min. The reason was that a much higher perfume dosage was used, and a split ratio was set to avoid oversaturation of the analytes. Other GC and MS conditions were kept the same as described in section 2.2.4.

3.3 Results and discussion

3.3.1 Method optimisation

3.3.1.1 Incubation temperature

In the same manner as the neat shampoo samples analyses, the incubation temperature of the rinsed hair swatch affects the amount of VOCs accumulating in the vessel headspace. Referring to equation (5), the partition coefficient of the analytes between the sample and headspace is reduced with higher temperatures. Again, the incubation temperature was selected to be 30°C to prevent the incomplete removal of moisture content in the TD tube that could cause crystal formation in the CIS during cryo-trapping of analytes, and lead to mechanical damage of the GC. In addition, the incubation temperature and the temperature during extraction should be kept close to room temperature, in this case at 30°C, to mimic real-life situations after hair washing. In most hair sensory evaluation studies, the temperatures ranged from 22°C to body temperature.[143-144] The RSDs, where n=3, ranged from 18.1% (allyl cyclohexylpropionate) to 52.3% (hexyl salicylate). As with previous deposition on rinsed-off hair studies[138-139], the largest source of error that may explain the high %RSD values observed could be the washing of hair swatches as it was almost impossible to reproduce the manual procedure perfectly during each washing despite the greatest effort to do so.

3.3.1.2 Incubation time

Since the amount of PRMs deposited on the hair swatch after rinse-off would possibly be minute, it is vital to accurately determine the incubation time, so as to provide the VOCs sufficient time to form an equilibrium in the vessel's headspace. At an incubation temperature of 30°C, the hair swatch was incubated in the DHS^{large} station for 30.00, 60.00, 90.00 and 120.00 min. **Figure 3.2** shows the logarithmic trend of the total mean peak areas obtained for perfume B while **Figure 3.3** shows the logarithmic trend of the mean peak areas obtained for all individual PRMs across the various incubation timings. Based on the total mean peak areas, it can be concluded that dynamic equilibrium had been achieved between the sample phase and the gaseous headspace after 60.00 min of incubation. Dihydromyrcenol took a longer time of 90.00 min to reach equilibrium probably due to its lower vapour pressure, while for benzyl acetate, it could be due to its low concentration originally present in perfume B. However, these observations could also be due to the error arisen from the washing procedure. The RSDs, where n=3, ranged from 18.1% (allyl cyclohexylpropionate) to 52.3% (hexyl salicylate). Again, the high RSDs could be due to the washing of hair swatches.

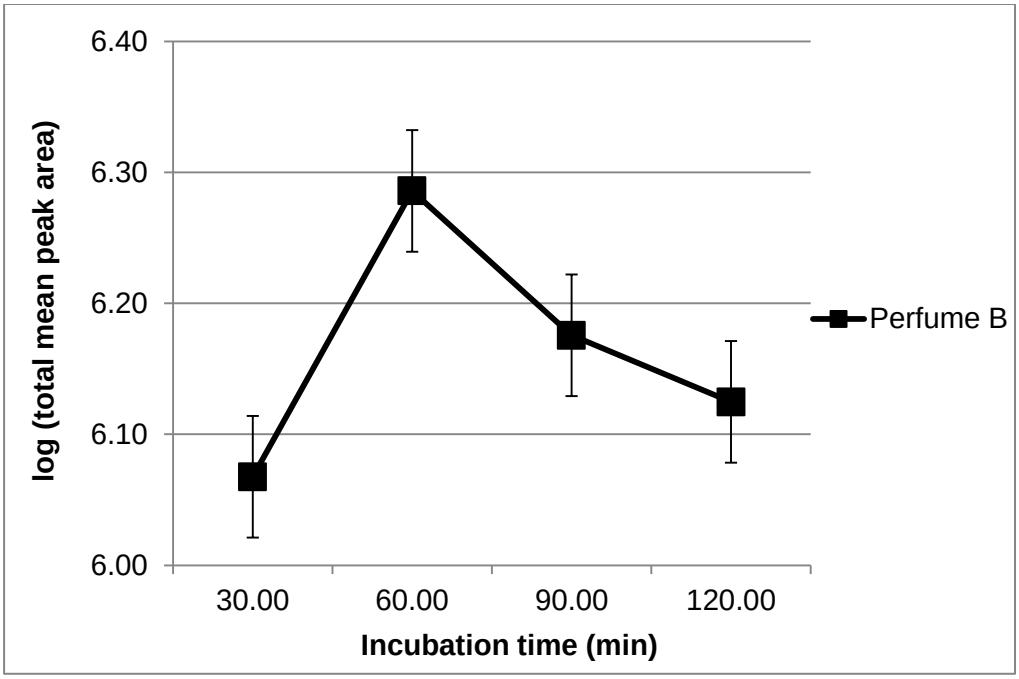

Figure 3.2 Logarithm of total mean peak areas of perfume B against incubation time in triplicate analysis.

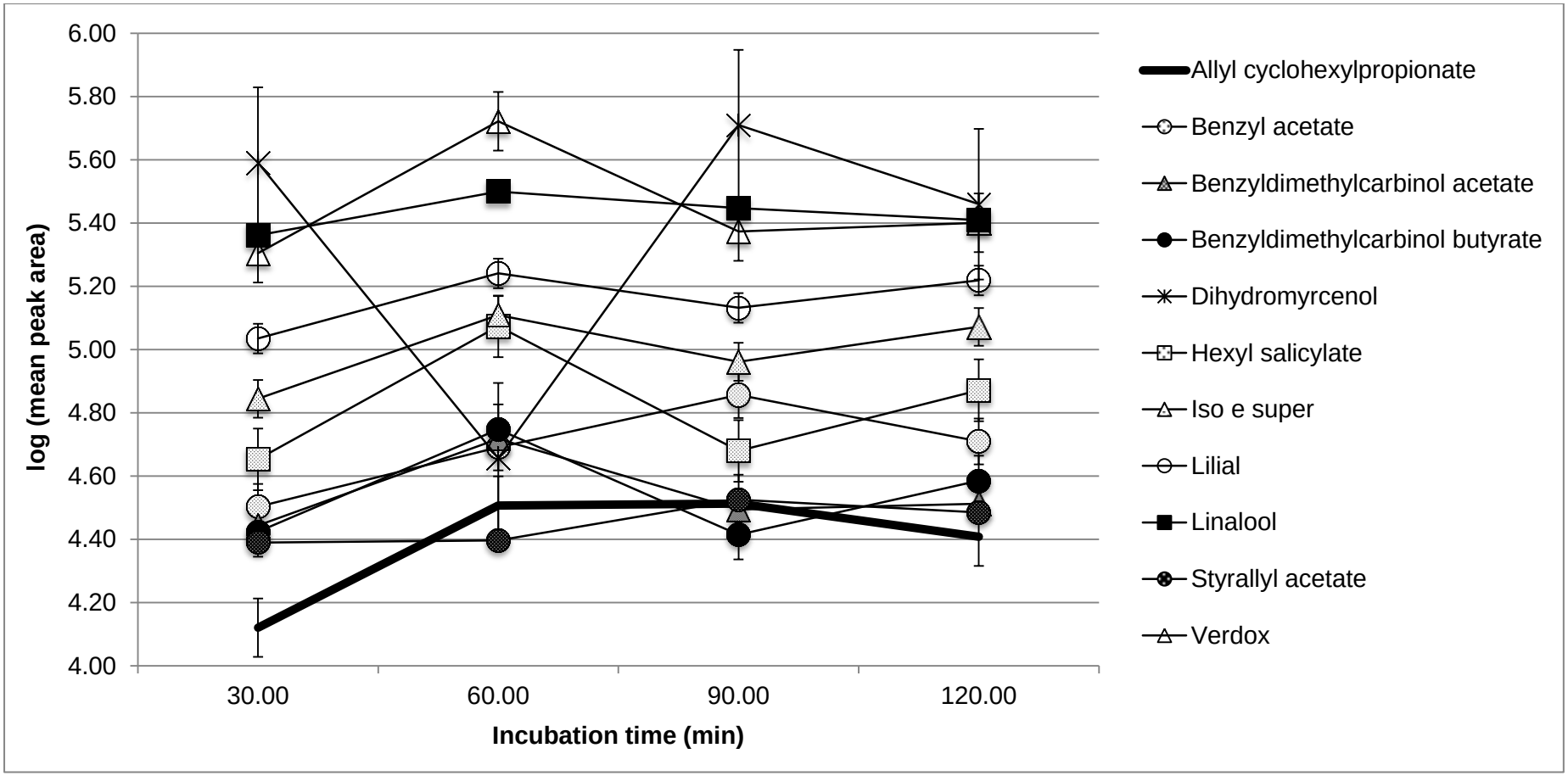

Figure 3.3 Logarithm of mean peak areas of perfume raw materials (PRMs) against incubation time in triplicate analysis.

3.3.1.3 Trapping phase purge volume

As previously explained in Chapter 2, higher amounts of analytes were recovered with increased purge volumes. **Figure 3.4** presents the logarithmic trend of the total mean peak areas obtained for perfume B whereas **Figure 3.5** illustrates the logarithmic trend for all individual PRMs across the different trapping phase purge volumes set. From the total mean peak areas, 3000.0 mL of purge volume was chosen as a compromise between analysis time and extraction efficiency. The decrease in peak areas for benzyl acetate and styrallyl acetate at 5000.0 mL was possibly caused by the excess purging that led to the removal of these materials, which were present in much lower concentrations as compared to other PRMs in the perfume. Moreover, the adsorption sites were mostly occupied by the high concentrations of moderate volatile compounds such as dihydromyrcenol, linalool and verdox. Likewise, these observations could also be due to the large error arisen from the washing procedure that triggered an exceedingly high RSD, where n=3, ranged from 33.1% (iso e super) to 120.0% (allyl cyclohexylpropionate).

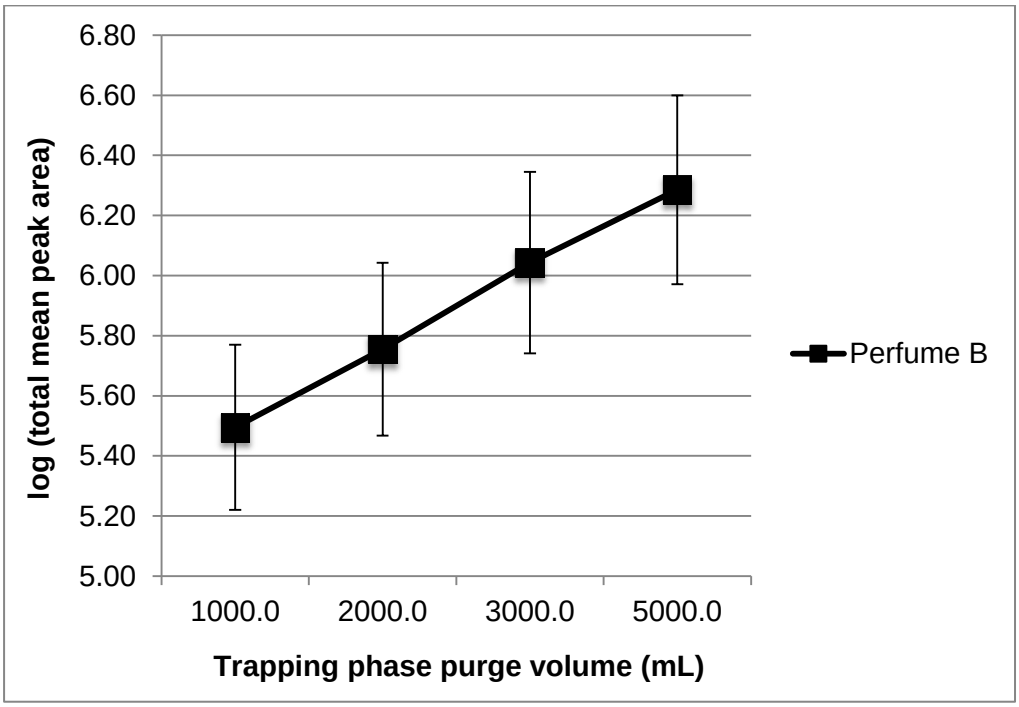

Figure 3.4 Logarithm of total mean peak areas of perfume B against trapping phase purge volume in triplicate analysis.

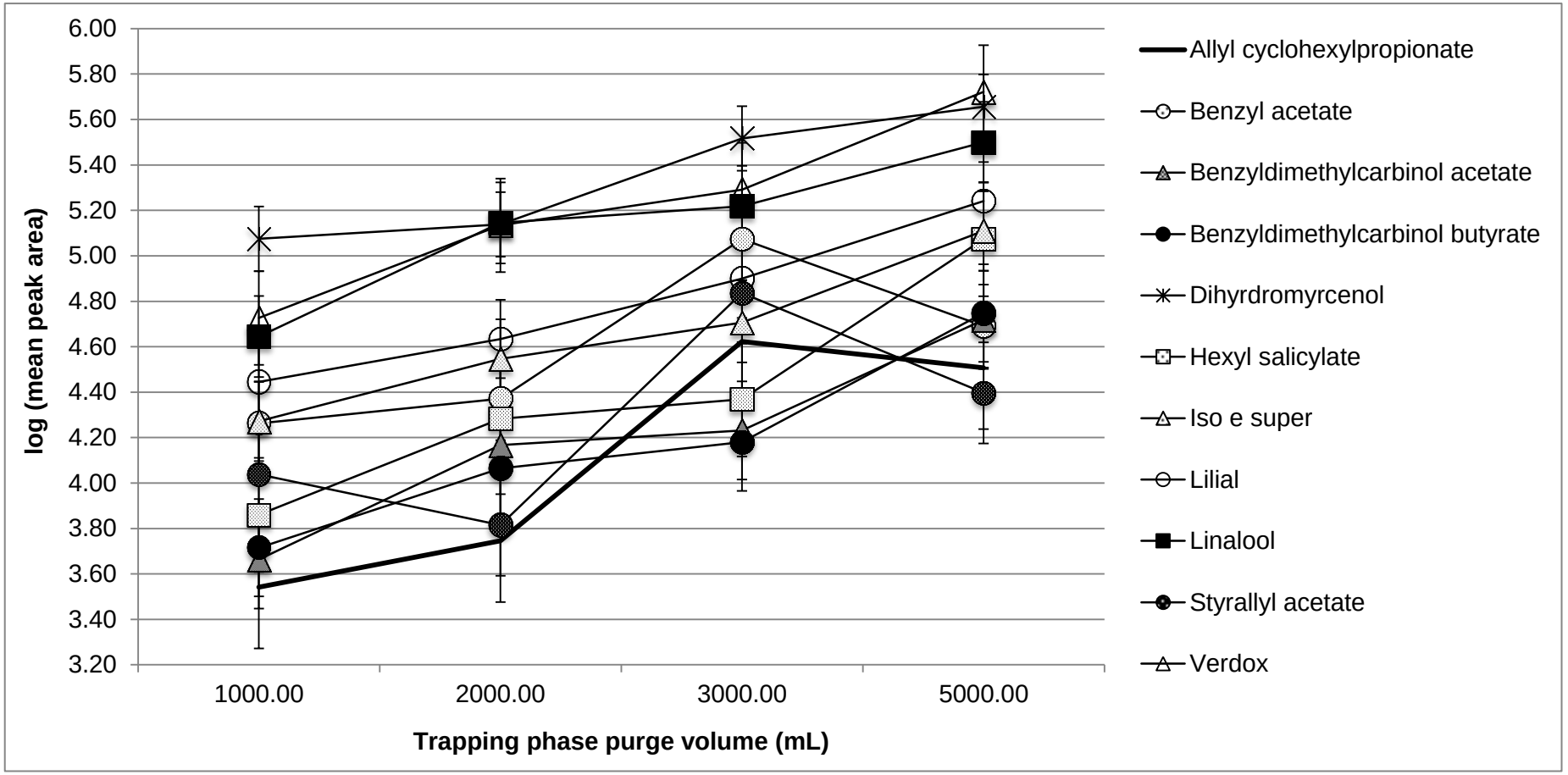

Figure 3.5 Logarithm of mean peak areas of perfume raw materials (PRMs) against trapping phase purge volume in triplicate analysis.

3.3.1.4 Trapping phase purge flow

Figure 3.6 exhibits the general logarithmic trend for the total mean peak areas for perfume B with the various trapping phase purge flow rates. The mean peak area achieved an optimum at 65.0 mL/min, followed by a decrease till 80.0 mL/min and remained almost constant at 100.0 mL/min. The logarithm of the mean peak areas for most PRMs also followed this trend, as shown in **Figure 3.7**. From this observation, the analytes might have experienced breakthrough sampling, such that a portion of analytes was lost before the completion of extraction, similar to the observations made by Zapata et al.[145] High flow rates of the purging gas prevented the analytes to interact sufficiently with the adsorbent and be retained. Two anomalies were noticed for benzyl acetate and dihydromyrcenol, where there was a sharp decrease in their peak areas from 50.0 mL/min to 65.0 mL/min before an increase again, which could only be attributed to the washing process. Eventually, 100.0 mL/min was chosen as the flow rate for future experiments as a compromise between analysis time and extraction efficiency. The RSDs, where n=3, ranged from 7.2% (hexyl salicylate) to 56.0% (benzyldimethylcarbinol acetate).

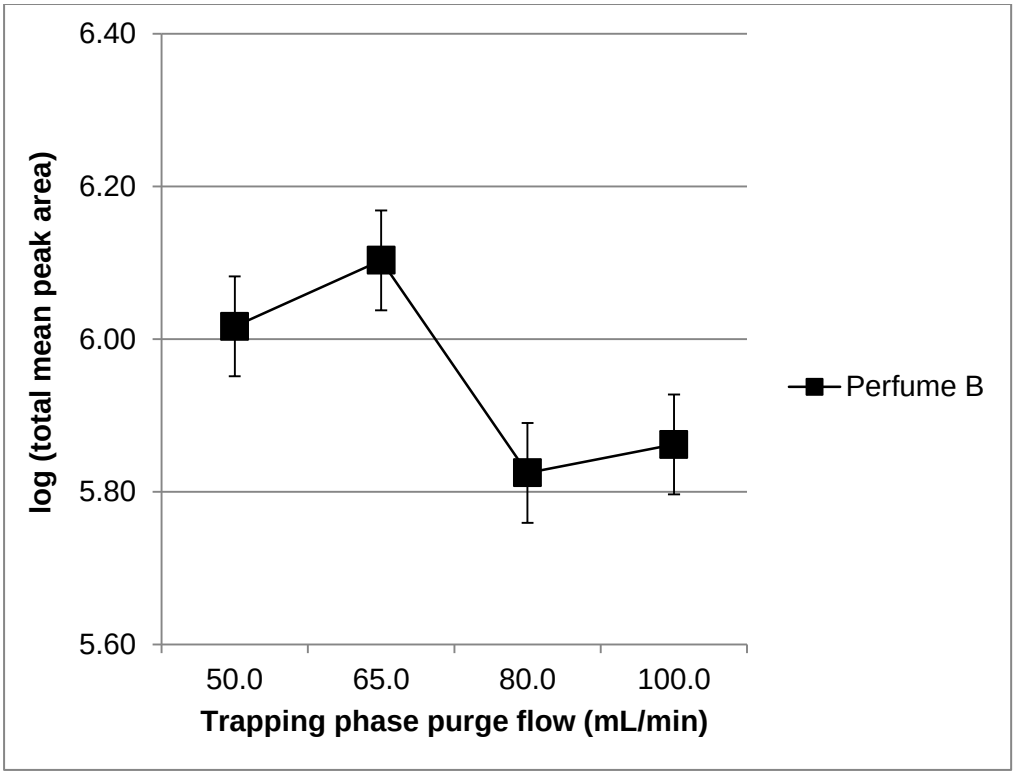

Figure 3.6 Logarithm of total mean peak areas of perfume B against trapping phase purge flow in triplicate analysis.

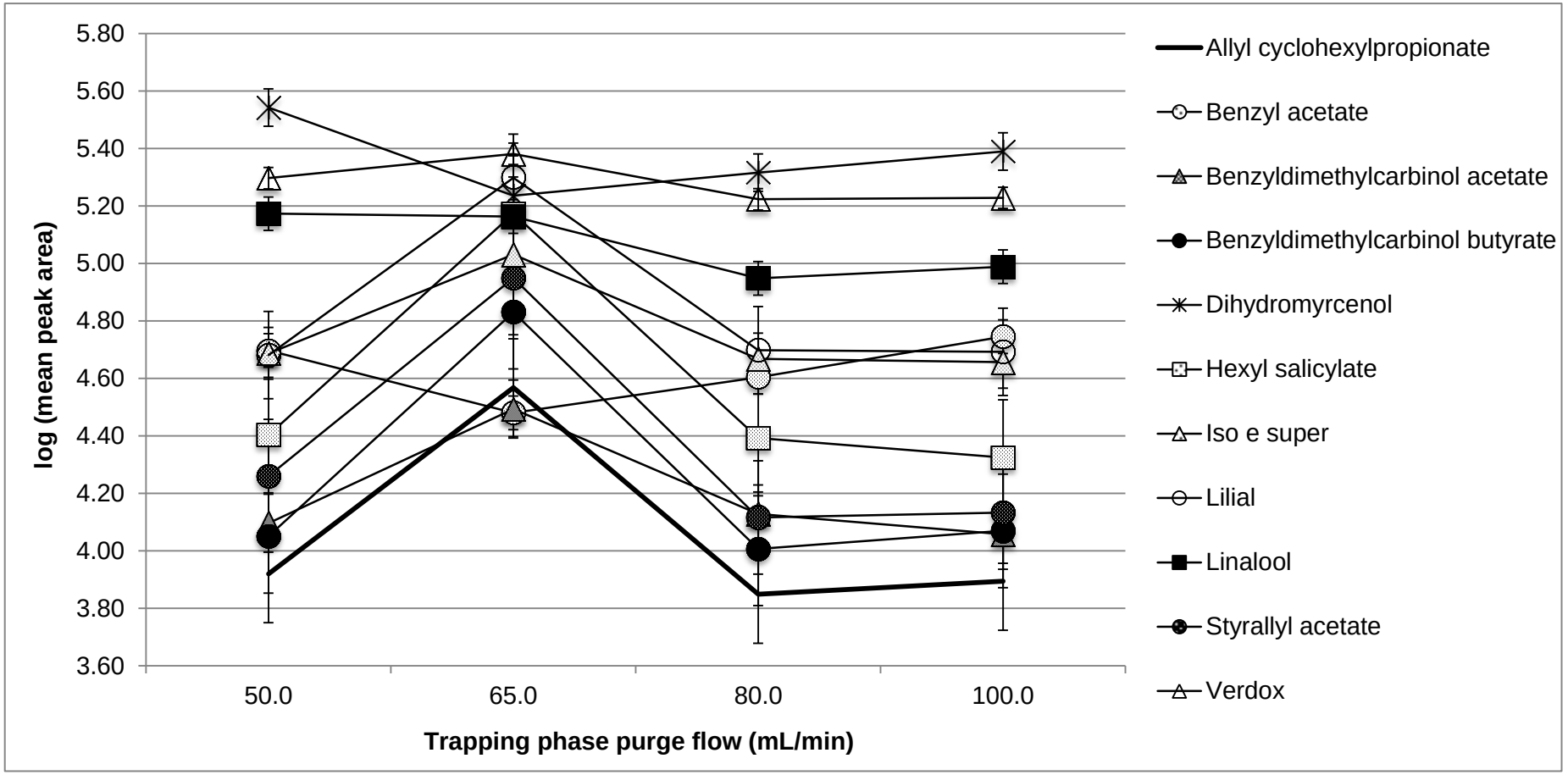

Figure 3.7 Logarithm of mean peak areas of perfume raw materials (PRMs) against trapping phase purge flow in triplicate analysis.

3.3.1.5 Drying phase purge volume

To optimise the drying phase purge volume, several volumes were experimented. Expectedly, the lowest purge volume at 500.0 mL gave rise to the highest total mean peak areas of perfume B. The total mean peak areas decreased sharply at 1000.0 mL and remained almost constant at higher purge volumes. This logarithmic trend is illustrated in **Figure 3.8**. A low dry purge volume was beneficial because it was sufficient to remove any moisture present without causing any crystal formation in the CIS, shortened the drying time needed and the signal intensities were not compromised. At high dry purge volumes, target analytes might have been purged out of the adsorbent, which would explain the drop in peak areas. Generally, the logarithm of the mean peak areas of all PRMs followed this trend as shown in **Figure 3.9**. The RSDs, where n=3, ranged from 7.2% (hexyl salicylate) to 56.0% (benzyldimethylcarbinol acetate).

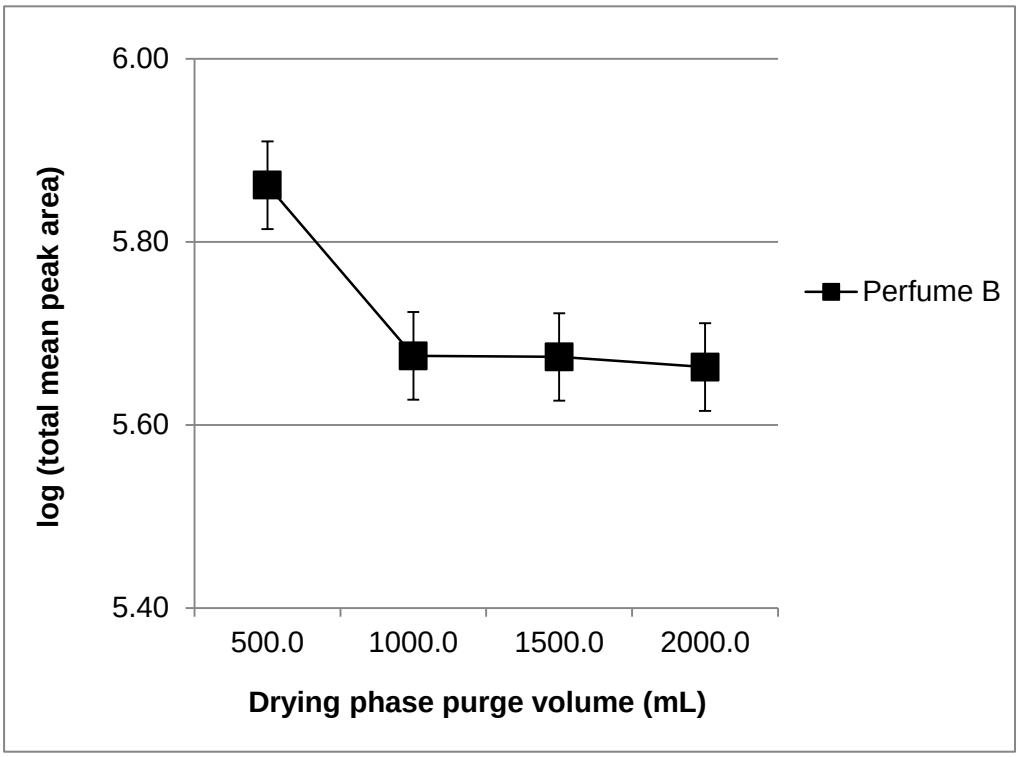

Figure 3.8 Logarithm of total mean peak areas of perfume B against drying phase purge volume in triplicate analysis.

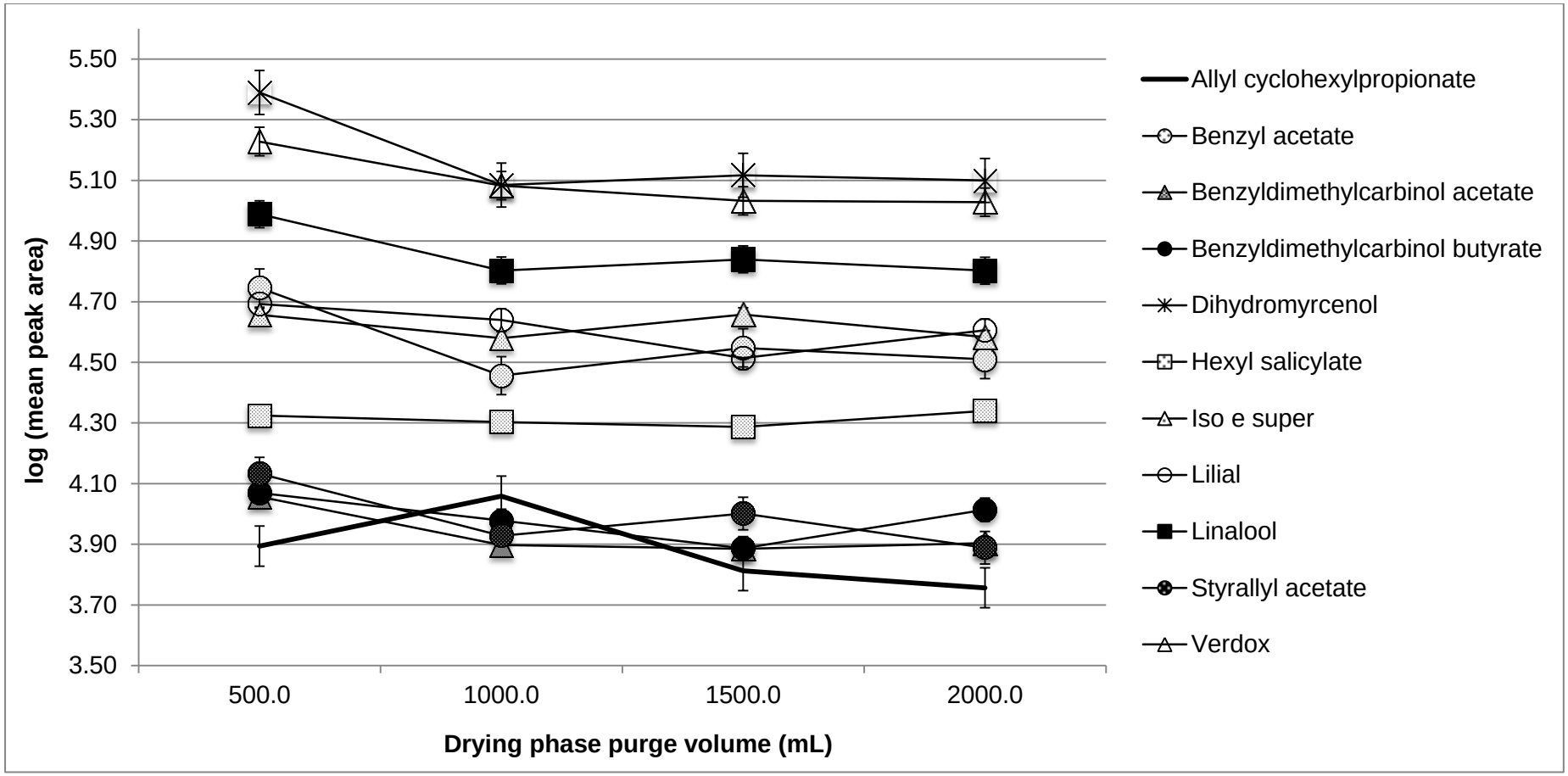

Figure 3.9 Logarithm of mean peak areas of perfume raw materials (PRMs) against drying phase purge volume in triplicate analysis.

3.3.2 Method validation

3.3.2.1 Linearity

Three calibration curve sets of the same concentration range were created. Each set of calibration samples was prepared by spiking directly onto 3 hair swatches, with perfume B at 0.0005%, 0.004% and 0.008% dosage levels. Following that, the hair swatches were extracted by LVDHS under the optimised conditions stated in section 3.2.4. The calibration curves were plotted using the total peak areas of perfume B against the various perfume dosages. **Figure 3.10** presents the calibration curves for set 1, with a R^2 value of 0.9988. The calibration set was duplicated on a different day as shown in **Figure 3.11**. A R^2 value of 0.9980 was derived. Lastly, the calibration set was prepared again on a third day. The calibration plot, as displayed in **Figure 3.12**, had a R^2 value of 0.9949. **Table 3.2** shows a good linearity achieved in all 3 studies on 3 different days with the individual R^2 of all analytes not lesser than 0.9516.

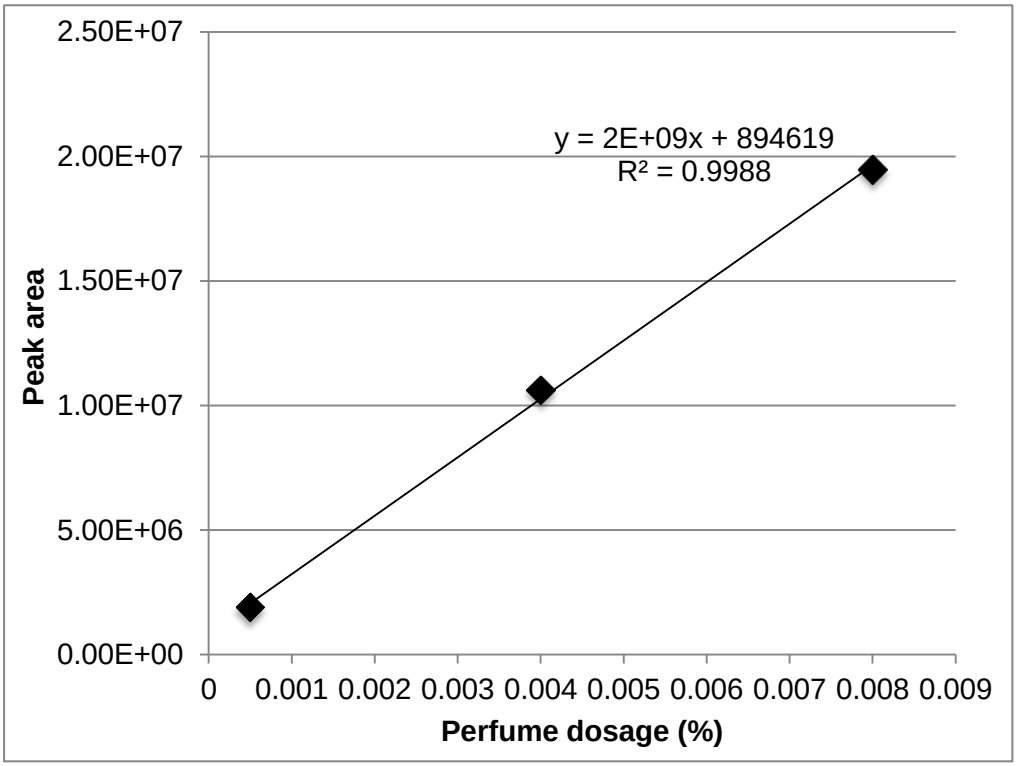

Figure 3.10 Set 1 calibration curve of 3 hair swatches spiked directly with perfume dosages of 0.0005%, 0.004% and 0.008%.

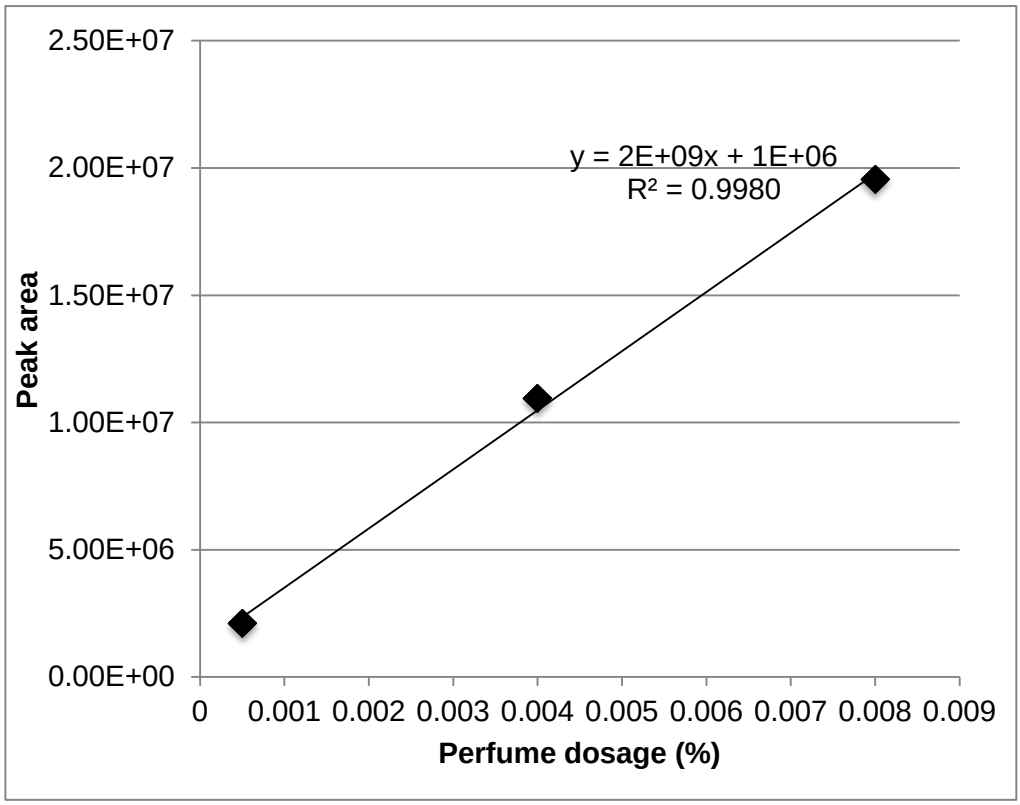

Figure 3.11 Set 2 calibration curve of 3 hair swatches spiked directly with perfume dosages of 0.0005%, 0.004% and 0.008%.

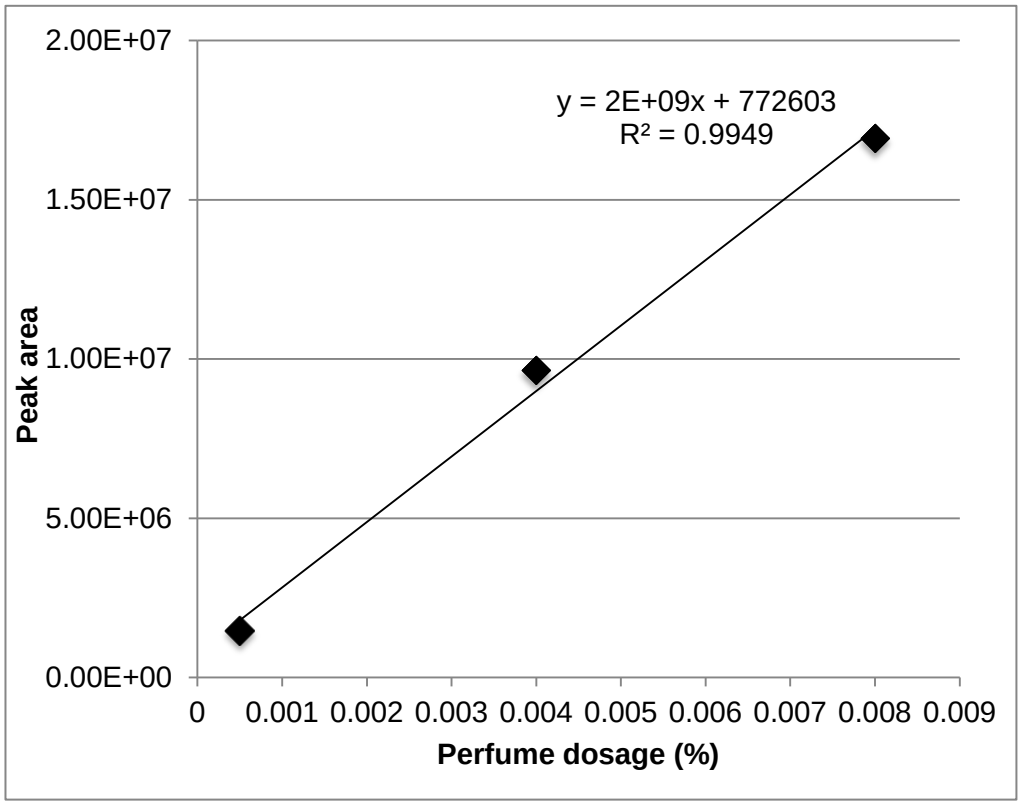

Figure 3.12 Set 3 calibration curve of 3 hair swatches spiked directly with perfume dosages of 0.0005%, 0.004% and 0.008%.

Table 3.2 Calibration linearity for all perfume raw materials (PRMs) derived from 3 calibration sets performed on 3 different days.

PRM	R^2		
	Set 1	Set 2	Set 3
Dihydromyrcenol	0.9898	0.9941	0.9722
Linalool	0.9904	0.9989	0.9782
Benzyl acetate	1.0000	0.9657	0.9982
Styrallyl acetate	0.9994	0.9966	0.9998
Verdox	1.0000	0.9844	0.9996
Benzyldimethylcarbinol acetate	0.9980	0.9960	0.9931
Allyl cyclohexylpropionate	0.9895	0.9984	0.9861
Benzyldimethylcarbinol butyrate	0.9829	0.9963	0.9776
Lilial	0.9745	0.9914	0.9743
Iso e super	0.9704	0.9721	0.9700
Hexyl salicylate	0.9532	0.9516	0.9733

3.3.2.2 Repeatability

The method's precision was determined by inter-day repeatability study using the 3 calibration sets at 3 different perfume dosages as stated in section 3.3.2.1. Each set of samples was prepared by spiking directly onto 3 hair swatches, with perfume B at 0.0005%, 0.004% and 0.008% dosage levels. After that, the hair swatches were extracted by LVDHS under the optimised conditions stated in section 3.2.4. Excellent RSDs were achieved for the samples spiked with 0.004% and 0.008% perfume dosages, with the highest at 14.8%. However, the RSDs for 0.0005% perfume dosage were unsatisfactory, with the highest at 35.2%. This could be attributed to the low concentration of analytes, which were only slightly above the baseline noise, hence resulting in a large source of error. All precision results are given in **Table 3.3**.

3.3.2.3 Sensitivity

The method's sensitivity was validated by its LOD and LOQ, which were derived using the calibration sample spiked with 0.004% perfume B. The LOD and LOQ of all PRMs were estimated by a S/N ratio of 3 and 10 respectively. The lowest LOD and LOQ deduced were found to be that of dihydromyrcenol, at 0.2 µg g^{-1} and 0.5 µg g^{-1} respectively. Again, the LOD and LOQ for benzyldimethylcarbinol acetate and hexyl salicylate could not be determined due to co-elution with the minor isomer of verdox and iso e super respectively. The LOD and LOQ of all PRMs are provided in **Table 3.3**.

Table 3.3 Precision of perfume raw materials (PRMs) spiked with 0.0005%, 0.004% and 0.008% dosages. Limits of detection and quantification determined by 0.004% dosage.

PRM	Inter-day precision (%RSD, n=3)			LOD (µg g^{-1})	LOQ (µg g^{-1})
	0.0005%	0.004%	0.008%		
Dihydromyrcenol	10.51	1.81	6.04	0.2	0.5
Linalool	13.51	4.11	8.35	0.3	1.0
Benzyl acetate	21.32	11.82	9.63	0.3	1.1
Styrallyl acetate	18.60	9.80	11.27	0.2	0.7
Verdox	22.68	13.41	5.50	0.2	0.8
Benzyldimethylcarbinol acetate	28.51	12.43	6.77	-	-
Allyl cyclohexylpropionate	31.21	10.23	8.76	0.8	2.8
Benzyldimethylcarbinol butyrate	29.43	11.16	9.07	1.5	4.9
Lilial	31.41	10.84	12.50	3.7	12.2
Iso e super	30.14	9.02	9.92	4.4	14.7
Hexyl salicylate	35.18	9.20	14.81	-	-

3.3.3 Analysis of rinsed-off hair swatches

The newly developed LVDHS method was applied to measure directly the perfume deposition on hair swatches after rinse-off. Seven hair swatches were each washed with 5.0 g of unperfumed shampoo base, which was spiked with perfume B at a predetermined dosage of 2.00%, according to the procedure stated in section 3.2.2. All hair swatches were extracted by LVDHS under the optimised conditions stated in section 3.2.4. They were then externally calibrated and quantified by calibration curve 1. The dosage of perfume B recovered after the rinse-off was calculated by substituting the total peak area into the following linear equation from calibration curve 1:

$$dosage_b = \frac{(A_b - 894{,}619.36)}{2{,}342{,}956{,}952.66} \quad (7)$$

where:
A_b is the total peak area of perfume B from the chromatogram
$dosage_b$ is the dosage of perfume B deposited on hair swatch in %

The percent deposition, % deposition, of perfume B on the hair swatch was then derived from dividing the dosage obtained from equation (7) by the initial 2.00% perfume dosage in the shampoo sample, as represented in the following equation:

$$\%\ deposition = \left(\frac{dosage_b}{2.00}\right)\ \% \quad (8)$$

The perfume dosages and the percentage deposition of perfume B deposited on all 7 hair swatches are presented in **Table 3.4**.

Table 3.4 Perfume dosages and percentage deposition of perfume B deposited on hair swatches.

Hair swatch	1	2	3	4	5	6	7
Perfume dosage deposited ($10e^{-05}$)	67.2	64.3	43.9	151	12.9	8.64	9.74
% deposition ($10e^{-03}$)	33.6	32.2	21.9	75.3	6.46	4.32	4.87

The percentages of perfume deposition were different across the 7 rinsed-off hair swatches, despite the same amount of starting material for all samples. Also, the deposition of each PRM fluctuated to a great extent as observed in the logarithm of peak areas (**Figure 3.13**). Expectedly, this proved the non-reproducibility of the washing procedure of the hair swatches in contrast to the good repeatability achieved by direct dosing of perfume on the hair swatches performed in section 3.3.2.2.

Previous studies presented the idea that heavier molecules were more likely to be deposited on the hair after rinsed-off, while more volatile molecules with low *log* P values tended to be rinsed off and lost during the drying period.[131, 146] Results from this study agreed with this hypothesis, where the amount of lighter and more volatile PRMs such as dihydromyrcenol, linalool and benzyl acetate were not able to be quantified accurately as their deposition dosages fell below the lowest calibration point of 0.0005%. Styrallyl acetate, on the other hand, being slightly heavier and having a higher *log* P value than benzyl acetate, deposited within the calibration range for all 7 hair swatches.

Heavier molecules with higher *log* P values such as allyl cyclohexylpropionate, benzyldimethylcarbinol butyrate, lilial, iso e super and hexyl salicylate were also well deposited on all 7 hair swatches. Of which, the dosage depositions of allyl cyclohexylpropionate, benzyldimethylcarbinol butyrate and lilial fell within the calibration range, thus were quantified accurately. For iso e super and hexyl salicylate, the dosage depositions were higher than the highest point in the calibration curve, 0.008%. However, the

highest calibration point was not further increased to a higher perfume dosage as competition on adsorption sites between these heavier, less volatile molecules and the lighter, more volatile molecules were noticed, as the peak areas of the following PRMs started to decrease at 0.01% perfume dosage (**Figure 3.14**); namely, verdox, benzyldimethylcarbinol acetate, allyl cyclohexylpropionate, benzyldimethylcarbinol butyrate, lilial, iso e super and hexyl salicylate. Therefore, the quantification of these 2 PRMs was based on extrapolation of the calibration curve.

Some other factors that may account for the differences in the deposition of chemicals are their binding affinities to the hair keratin, and their diffusabilities into the hair. In fact, the binding affinity to hair keratin is also governed by a number of factors. For instance, the size of the chemical molecule, the isoelectronic point of the hair and other substances in the shampoo formulation.[147] These factors could all contribute to the competitive binding of ingredients to the hair keratin, which consists of limited binding sites.[141] Further studies concerning hair keratin ought to be conducted to understand the deposition of chemicals associated with their attraction to the hair keratin.

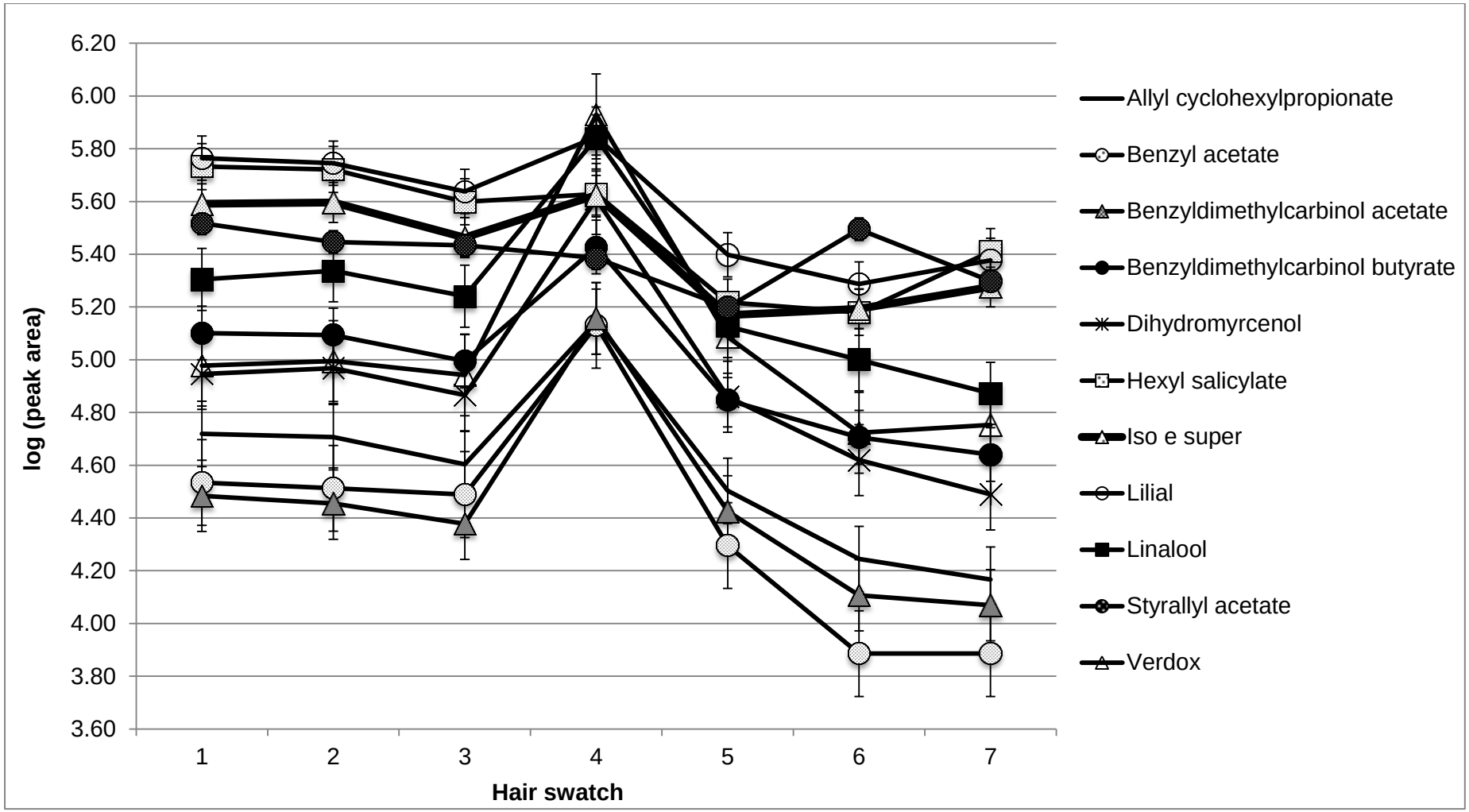

Figure 3.13 Logarithm of peak areas of perfume raw materials (PRMs) deposited on hair swatches after rinsed-off.

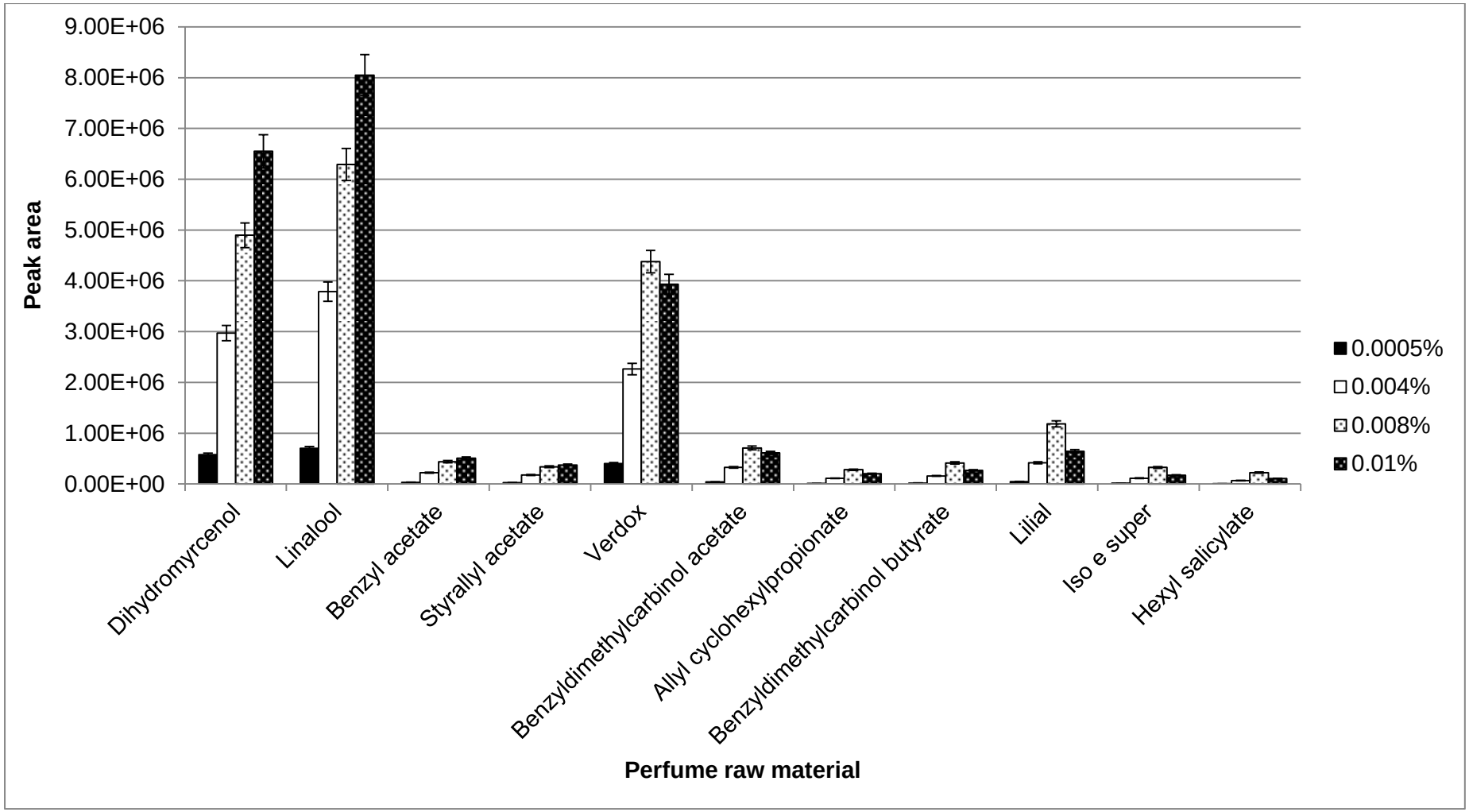

Figure 3.14 Peak areas of perfume raw materials (PRMs) for various perfume dosages of calibration curve.

3.4 Conclusion

An innovative and non-destructive automated LVDHS-TD-GC-MS method was developed in this experiment to determine the amount of perfume deposited on hair swatches after rinsed-off. The main parameters optimised were the incubation temperature, incubation time, trapping phase purge volume and flow, and the drying phase purge volume. This solvent-less technique was a supplementary technique to the DHS-TD-GC-MS, where larger applied samples can be analysed based on DHS extraction, in addition to the neat consumer products. The newly developed method was validated, and exhibited high linearity in 3 target concentrations. Reasonable repeatability was also attained, alongside excellent sensitivity as represented by its LOD and LOQ. Lastly, the method was applied to measure the perfume deposition directly from hair swatches after rinsed-off, where it clearly provided an insight into the deposition characteristics of different PRMs. An understanding of the deposition extent of each PRM would be useful to a perfume's knowledge for future perfume creations, which can affect the consumer's choice during the selection of a product and its brand.

Chapter 4. General conclusions, recommendations and future work

Constant exploration and development of advanced efficient analytical techniques are vital for quantitative studies of flavours and fragrances volatiles. In this research, 2 analytical methods were developed and validated for the quantification of flavours and fragrance volatiles in consumer products, and deposition of fragrance volatiles on hair.

In the first study, a solvent-less, rapid and sensitive automated DHS-TD-GC-MS method was first optimised through the measurement of perfume volatiles in neat shampoo samples. Minimal sample preparation steps were required, and the important DHS parameters optimised were the DHS incubation temperature, incubation time, trapping phase purge volume, trapping phase purge flow, sorbent material and the drying phase purge volume. Following the parameters optimisation, the method was then validated for its linearity, repeatability and sensitivity where desirable results were achieved in all aspects. To further demonstrate the applications of this method, it was used to quantify neat shampoo samples with different spiked dosages where the experimental and target dosages deduced were relatively close, which demonstrated the method's accuracy.

Further applications included the quantification of flavour volatiles in selected common food products, green tea and milk flavour. Ten ingredients were identified in the green tea samples, with the highest RSD at 13.7%, while twelve ingredients were identified in the milk flavour samples, with the highest RSD at 16.1%. In short, the DHS-TD-GC-MS proved to be a reliable and convenient method for measuring volatiles in different sample matrices. However, it is also a method that is sensitive to temperature. Therefore, it would be recommended to pump a water/ethanol mixture coolant to the DHS system to maintain it at approximately room temperature at all times after sample extraction.

Further, some samples contain high water content especially liquid samples. Water condensation can occur in the sample vial for these samples and moisture might not be entirely removed by the drying step and solvent

venting during thermal desorption. Moisture content in the sample can cause crystal formation in the CIS, which damages the GC. A commercially available stainless steel wire mesh with PTFE coating that allows volatiles to penetrate without water passing through could be considered to be inserted before the analytes enter the GC as an additional step to prevent water from entering the system. Alternatively, a similar water trap containing lithium chloride on a porous support, which is heated after every analysis through back flushing, developed by Kolb et al.[148] could be used.

In the second study, a further development of the DHS-TD-GC-MS method was accomplished. The area of direct fragrance measurement on substrates with a non-destructive method has not been researched upon. Therefore, the novel LVDHS-TD-GC-MS method was developed to determine the amount of perfume deposited on hair swatches after rinsed-off. The main benefits of this technique were its ability to measure directly the deposition from the sample non-destructively, the simple solvent-less sample preparation steps and high sensitivity. The parameters optimised in this study were similar to the DHS-TD-GC-MS and the method was also subsequently validated for its linearity, repeatability and sensitivity. Satisfactory results were obtained for method validation.

The method was then applied to measure actual perfume deposition on hair swatches that were rinsed off with shampoo samples of a predetermined dosage. The fluctuations in the total amount of perfume deposited across different samples explained the non-reproducible washing procedure. The washing procedure consistency could be improved by using an automated hair-washing machine. Another objective of this experiment that was fulfilled was to gain deeper knowledge of PRMs that were more substantive to hair substrates as compared to others. The building of such knowledge could allow industry professionals to develop high performance and cost efficient perfumes in products that consumers would likely prefer. Due to the limited availability of the instrument, applications on other sample matrices were not attempted. However, it is highly possible to apply this method to a large number of different samples, or even samples with encapsulation technology. One example would be meat samples to monitor flavour volatiles since many, if not all, methods require the samples to be cut.

In conclusion, the development of the above-mentioned novel methods allowed the successful monitoring and quantification of flavours and fragrance volatiles in both neat and applied samples. It is believed that these methods can then be utilised conveniently and, accurately in a wide variety of sample matrices where volatiles measurements are required.

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